Reverse Translational Research

How Clinical Trials on Fluorescence Imaging for Vocal Cord Cancer Fuels Fundamental Research

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Abstract: Translational research consists in translating fundamental research results as closely as possible to patients. Researchers sometimes underestimate these studies because it is thought that, although essential for setting up new investigation tools, they do not deepen fundamental knowledge. However, users face specific difficulties due to the variability of the biological systems under study. Variability is easily understood from one patient to another, but there is also variability in a single patient whose metabolism evolves together with therapeutic actions. Results obtained in translational research often depend on this variability, and new questions and scientific obstacles arise when research is applied to the real world. In order to address these new challenges, reverse translational research is required. Fundamental research is fuelled by the results of translational research. In this position paper, we consider vocal cord fluorescence imaging as an example of bi-directional translational research. First, we briefly recall the basics of fluorescence imaging, and we explain why commercial fluorescence systems lead to variable estimations of their efficiency by end-users. Second, we describe solutions intended to improve fluorescence techniques. This position paper will then make conclusions.

1 INTRODUCTION

Translational research is a fairly new and rapidly evolving concept. The general idea is to translate fundamental research results as closely as possible to patients *via* pre-clinical and clinical trials. In other words, it consists in taking research from bench to bedside. Sometimes researchers underestimate these studies because it is thought that, although essential for setting up new investigation tools, they do not deepen fundamental knowledge. However, users face specific difficulties due to the variability of the biological systems under study. Variability is easily understood from one patient to another, but there is also variability in a single patient whose metabolism evolves together with therapeutic actions. Results obtained in translational research often depend on this variability, and new questions and scientific obstacles arise when research is applied to the real world. In order to address these new challenges, reverse translational research is required. Fundamental research is then fuelled by the results of translational research.

Consequently, the concept of bidirectional translational research is emerging: forward from researchers to end-users and then back to research to answer new questions or improve current results. In this position paper, we illustrate this idea through examples concerning fluorescence optical diagnosis, and more precisely tools that could be used to diagnose vocal fold disease.

In part two of this paper, we briefly recall the basics of fluorescence and we highlight the fact that both the excitation wavelength and observation wavelength window must be carefully chosen to efficiently assess the composition of the tissue under examination. This section illustrates what is called translational research.

In part three, we explain why end-user teams often disagree on the performances of current commercial fluorescence systems. Here, we will see that multimodality may offer versatile systems intended for most end-users. This section illustrates how translational research can put to evidence new

scientific obstacles.

Part four is devoted to the description of hyperspectral fluorescence techniques that may be used to improve diagnosis efficiency. We also propose architectures based on multimodality. Early experimental results will be presented for these techniques. This section illustrates how translational research fuels fundamental research by means of what we call reverse translational research.

This paper will then make conclusions.

2 BASICS OF FLUORESCENCE: TRANSLATION

One way to discriminate unhealthy tissues from healthy ones is by detecting an abnormal concentration of particular proteins by means of fluorescence measurements. Fluorescence can be explained with the help of the Jablonski diagram (figure 1). By illuminating the molecules, electrons absorb light and "go upward" from energy state U0 to energy state U1. The molecules then relax, first by a non-radiative loss of energy (from U1 to U2) and finally down to energy state U0. This last step is a radiative process and the light emitted at this stage is called fluorescence.
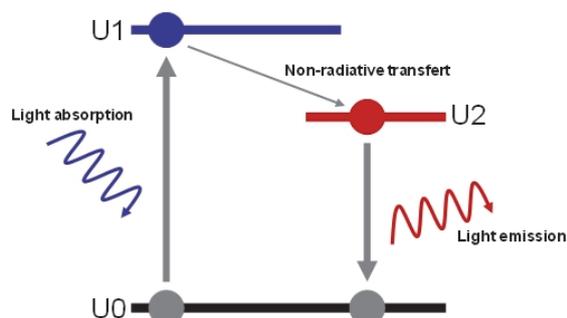

Figure 1: Jablonski diagram.

Two different methods can be used to detect proteins. The first deals with exogen fluorescence which consists in applying a mixture of mono- or polyclonal antibodies functionalized with fluorophores. This method requires foreign substances to be injected into the body, however.

The other way of detecting the target proteins is to use endogen fluorescence, also termed autofluorescence. It consists in studying the natural fluorescence of the target proteins. In this case, once the target proteins are determined, an illumination source suited to their absorption spectrum must be chosen. The first step of the study is therefore to determine the target proteins adequately. For instance, for vocal folds, we can name collagen, elastin, NADH, flavins or porphyrins. Figure 2 shows the absorption and emission spectra of flavins (Wagnieres 1998 – Richards-Kortum 1996).
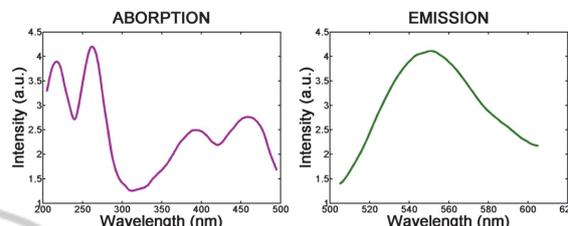

Figure 2: Absorption and emission spectra of flavins.

2.1 Influence of the Excitation Wavelength

Fluorescence intensity depends on different factors such as the quantum properties of the fluorescent protein, its concentration in the tissue and the absorption of both excitation and emission wavelengths by the tissue. These parameters are not easily controllable. However, fluorescence intensity also depends on the excitation wavelength used. Figure 3 illustrates this with flavins. The shape of the emission spectrum remains constant but its amplitude depends on the excitation wavelength.
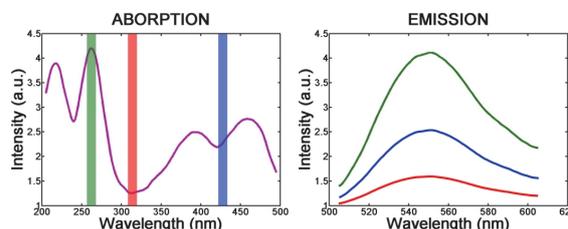

Figure 3: Effect of the excitation wavelength on fluorescence intensity.

This illustrates the importance of the choice of excitation wavelength. Note that the fluorescence intensity also depends on the spectral width of the excitation around the central excitation wavelength.

2.2 Influence of the Observation Wavelength Window

Several fluorescent proteins are usually present in tissue. A single excitation wavelength often induces fluorescence in different proteins even if the excitation wavelength is carefully chosen. This does not necessarily mean that observation provides average information on each individual protein

contribution. Since different proteins emit fluorescent light according to their own emission spectra, the choice of the observation wavelength window may help differentiate between the information from each protein. This is illustrated in figure 4 in the case where both flavins and porphyrins are considered (Wagnieres 1998 – Richards-Kortum 1996).
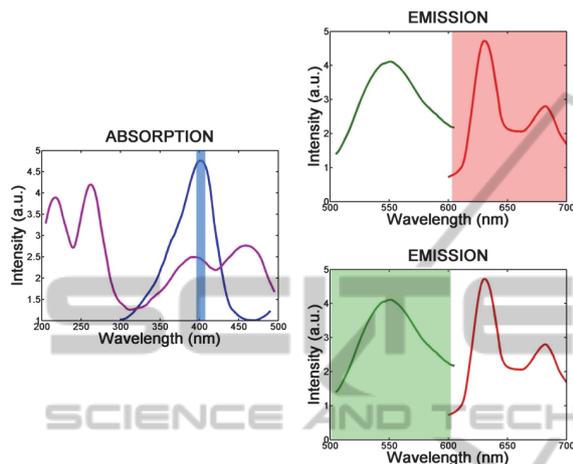

Figure 4: Influence of the observation window.

As can be seen, when higher observation wavelengths are considered, the greatest contribution comes from porphyrins. Conversely, when shorter wavelengths are considered, the greatest contribution comes from flavins.

3 TRANSLATIONAL RESEARCH: CONFRONTATION WITH REAL SITUATIONS

In real life, the situation is much more complicated due to the large number of fluorescent proteins present in the tissues. The figure below shows the excitation and emission spectra of several proteins (Wagnieres, 1998); (Richards-Kortum, 1996).

It can be seen that neither the choice of the specific excitation wavelength nor the choice of the observation window can help to dissociate the fluorescence contribution of each individual protein.

Concerning translational research, a large number of studies have been conducted in order to assess the efficiency of experimental or commercial devices. Highly interesting reviews have been published (Piazza, 2011); (Kraft, 2010); (Shin, 2010); (Mehrotra, 2010); (Rethman, 2010).
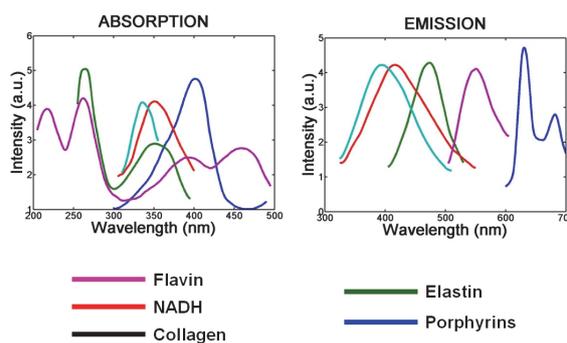

Figure 5: Absorption and emission spectra of some proteins.

Commercial apparatuses relying on this principle are readily available but the clinical trials performed to date do not present sufficient evidence of their ability to provide a reliable diagnosis (Piazza, 2011); (Rethman, 2010). These systems suffer from the fact that the excitation is made over a large wavelength band (Arens, 2007); (Mehrotra, 2010). In other words, many proteins are excited: the useful signal is buried in the various fluorescence signals emanating from non-relevant proteins, thus preventing the target protein from being detected. The same is true of their detection principle, as the observation wavelength range is also quite wide. Therefore, superimposition of information from a large number of proteins is observed and dilutes useful signal into what can be considered as noise. Figure 6 shows the excitation and observation wavelength windows commonly used in commercial systems.

Although autofluorescence is considered as highly effective in the early diagnosis of laryngeal cancer and its precursor lesions (Kraft, 2010), clinical trials lead to varying specificity and sensitivity results even with the same commercial system: 98% sensitivity and 100% specificity (Lane, 2006), 50% sensitivity and 38.9% specificity (Mehrotra, 2010) and 97% sensitivity and 94% specificity (Poh, 2006).
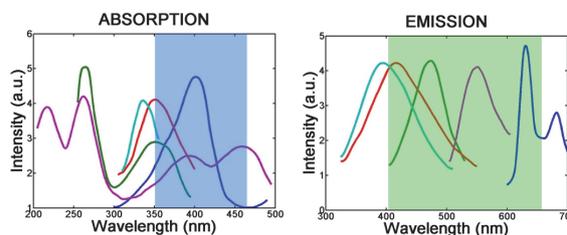

Figure 6: Wavelength windows commonly used in clinical trials.

In fact, in the case of upper aerodigestive tract cancer evaluation, optical techniques such as tissue staining, chemiluminescence, autofluorescence and tissue reflectance analysis have given unconvincing results. For the latter two techniques, the following description was reported (Rethman, 2010):

- *"There is insufficient evidence that commercial devices based on autofluorescence enhance visual detection of potentially malignant lesions beyond that achieved through a conventional visual and tactile examination (Patton, 2008).*

- *There is insufficient evidence that commercial devices based on tissue reflectance enhance visual detection of potentially malignant lesions beyond that achieved through a conventional visual and tactile examination (Patton, 2008)."*

The conclusion to be made regarding these translational research results is that none of the techniques is entirely satisfactory. Because these techniques can be greatly enhanced in terms of sensitivity and specificity, they often require subjective interpretation and depend on the visual recognition skills of the examiner (Shin 2010). The same authors also explain that *"the combination of wide-field and high-resolution fluorescence imaging systems with automated image analysis should be investigated to maximize overall diagnostic performance"*.

At this stage, forward translational research was conducted. Research on fluorescence led to the testing of advanced systems in clinical trials. These trials concluded that systems are largely improvable in terms of specificity and/or sensitivity. We have explained the reasons for information loss in the present paper. Reverse translational research should now be envisaged to explore the performance of advanced fluorescence techniques, possibly coupled with other optical investigations of tissue properties.

4 REVERSE TRANSLATIONAL RESEARCH: HOW TO ANSWER NEW OBSTACLES HIGHLIGHTED BY CLINICAL TRIALS

We entirely agree with (Shin, 2010) concerning the utility of combined techniques. Furthermore, we believe that other modalities may be included in advanced devices.

For example, we may consider a device that can excite target molecules at several specific wavelengths and also perform hyperspectral signal detection. We can thus analyze one or more small spectral bandwidths centered on the fluorescence wavelengths of several proteins (Muller, 2003); (Gillenwater, 1998).

4.1 Possibilities with Hyperspectral Fluorescence

Our basic experimental set-up consists of a fiber probe containing several optical fibers (figure 7: left). The central one is used to illuminate the tissue with a series of monochromatic wavelengths (*via* optical switches). Collection fibers are used to collect the emitted fluorescence. The light is then launched into a spectrometer. For each pixel of the image, the whole fluorescence spectrum is recorded as depicted in figure 7 (right) in the case of a tree leaf.
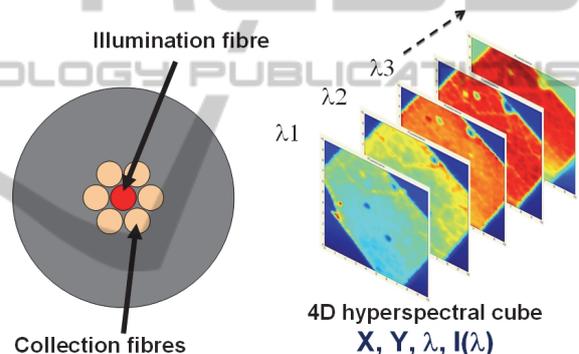

Figure 7: Optical fiber probe (left) and hyperspectral fluorescence image (right).

In our preliminary experiments, the image is formed by scanning the sample. In a more advanced system, fiber bundles coupled with hyperspectral CCD cameras will be employed.

The image obtained in this case consists of a 4D hyperspectral cube. There are different ways of investigating this hyperspectral cube. Figure 8 illustrates this. On the one hand we can select different observation wavelength windows (top right). It can be clearly seen that different pieces of information appear depending on the observation window. On the other hand, we can try to define hypervolumes in the hyperspectral cube. These hypervolumes can be specific to the possible pathological nature of the tissue. This can be achieved through the use of data processing algorithms, such as the Kernel Principal Component Analysis or the Support Vector Machine (Diaz-Ayil, 2007); (Adbat, 2012).
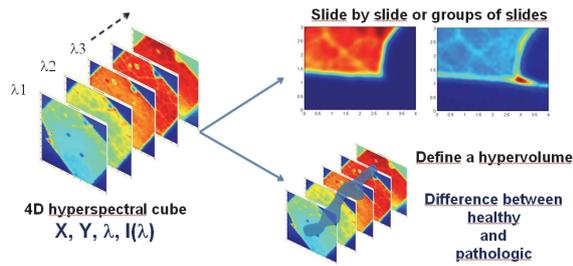
Figure 8: Different ways of exploiting hyperspectral fluorescence.

This hyperspectral technique can be further developed using images obtained with different excitation wavelengths. For each supplementary excitation wavelength, four dimensions are added to the hyperspectral cube as depicted in figure 9.
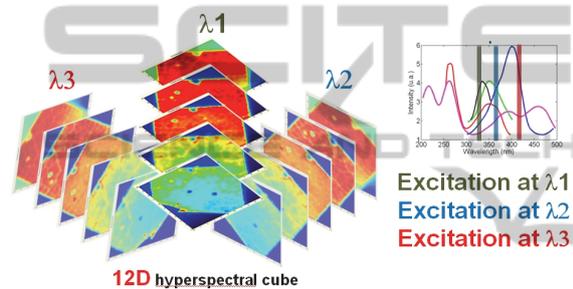

Figure 9: Illustration of multiple excitations and hyperspectral fluorescence imaging.

Note that fluorescence lifetime imaging could also be included in this type of measurement.

4.2 Other Possibilities with Diffusion

This autofluorescence measurement can also be coupled with an analysis of the diffuse reflectance of the tissues, *i.e.* on the photon elastic scattering process occurring in the biological sample. The bimodality of the system obtained can then significantly improve the sensitivity and specificity of the diagnosis (Diaz-Ayil, 2007). Beyond sheer data acquisition and direct analysis, further information can be obtained from the clinical trials by coupling and crossing the fluorescence and scattering measurements, allowing us to retrieve the intrinsic fluorescence characteristics of the sample (Wu, 1993); (Muller, 2001).

Figure 10 illustrates the possibilities offered by spectroscopic reflectance measurements. We can see that different details can be observed depending on the spectral observation window used to investigate diffusion properties of tissues. Note that these images were obtained with the same fiber probe as

the one used in the previous sub-section, thus demonstrating that multimodality can be obtained relatively easily.
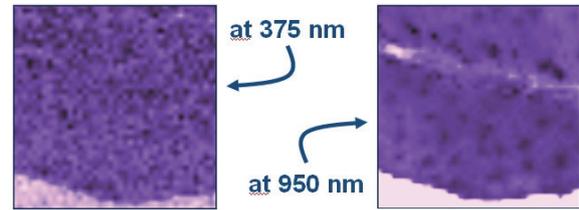

Figure 10: Example of spectral analysis of tissue diffusion.

Now let us go back to the discussion of translational research. In the previous section, we have seen that translational research highlights obstacles and questions that would not have arisen if they had not been applied to real clinical situations. In this section, we see that it is the role of reverse translational research to go back to more fundamental studies and to work at innovative solutions in order to address these new questions.

5 CONCLUSIONS

In this position paper we look at optical fluorescence imaging of the vocal folds as an example of bi-directional translational research. As mentioned in the introduction, translational research is sometimes underestimated because it is thought that it does not deepen fundamental knowledge.

However, users face specific difficulties due to the variability of the biological systems under study. Results obtained in translational research often depend on this variability and new questions and scientific obstacles arise when research is applied to the real world. In order to address these new challenges, reverse translational research is required. Fundamental research is then fuelled by the results of translational research and the latter should be considered essential to fully understand the biological system under study.

We focus herein on advanced fluorescence techniques in order to illustrate bidirectional translational research. The optical methods that are currently used are in need of improvement. We thus propose to develop a standalone device able to assess the possible pathological nature of vocal folds. Pre-clinical and clinical trials will then be conducted in order to transform the expected research results into new optical diagnosis tools.

To conclude, we believe that translational research should not be underestimated because it

fuels fundamental studies when new questions or scientific obstacles have been highlighted by pre-clinical or clinical trials. Through the example of vocal cord fluorescence imaging, this position paper illustrates that the concept of bi-directional translational research should be applied to all work aiming to develop new medical devices.

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