Perspective

Towards a minimally invasive sampling tool for high resolution tissue analytical mapping

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Abstract

Multiple spatial mapping techniques of biological tissues have been proposed over the years, but all present limitations either in terms of resolution, analytical capacity or invasiveness. Ren et al (2015 Nanotechnology 26 284001) propose in their most recent work the use of a picosecond infrared laser (PIRL) under conditions of ultrafast desorption by impulsive vibrational excitation (DIVE) to extract small amounts of cellular and molecular components, conserving their viability, structure and activity. The PIRL DIVE technique would then work as a nanobiopsy with minimal damage to the surrounding tissues, which could potentially be applied for high resolution local structural characterization of tissues in health and disease with the spatial limit determined by the laser focus.

Keywords: laser ablation, biopsy, biodiagnosis, scanning, mapping, biomedical imaging, high resolution

(Some figures may appear in colour only in the online journal)

Current advancements in nanotechnology are playing a key role in the development of new analytical tools in medicine and biomedical research. This allows for the high throughput screening of even very small biological samples enabling both a genomic and a proteomic research revolution [2–4]. However, while the analytical tools available are becoming more and more powerful, we still significantly rely on extraction methods from the last century. The highly invasive metal scalpel approach, including the use of punch biopsy tools, is only slowly being replaced in specific applications by laser scalpels especially where high precision and minimal risk of damage to the nearby tissues are stringent requirements. This is the case in ophthalmology [5–7], dermatology [8, 9], as well as in the treatment of distal parts of limbs [10] and in vascular endoscopic surgery [11]. However, the resolution of these techniques is still far below cellular-level surgery that the use of laser-based techniques could potentially allow. The general approach to laser-based incision and ablations is to locally deposit a significant amount of thermal energy to cause the melting/burning of tissues. The effects of the procedure extend beyond the limits of the focused beam and can damage tissue up to 800 μm past the intended cut border [12] effectively limiting the spatial resolution achievable. Recently, it has been shown that a picosecond infrared laser (PIRL) offers an optimized deposition process capable of reducing invasiveness and approaching an authentic cellular scale resolution [13]. This is achieved by tuning IR pulses to infrared active vibration, thus selectively energizing water molecules and ejecting the local tissue (ablation/cutting). The pulse duration is both (a) short enough to avoid thermal or acoustic transport to adjacent areas, and (b) long enough to avoid plasma formation and ionizing radiation. PIRL presented less invasiveness and...
overall less local damage when compared to a skin punch biopsy and to a conventional laser system on an in vivo model of mouse skin, easily achieving a damaged zone of less than 10 μm with minimal impact on the surrounding tissues [12].

Even more exciting than the obvious applicability of PIRL as a reliable microsurgery tool is the possibility to collect the tissue components ablated by the laser pulses for biochemical analysis and diagnostics. To this end, Ren et al [1] have applied PIRL under conditions of ultrafast desorption by impulsive vibrational excitation (DIVE) [14]. With picosecond laser pulses, water molecules are supplied with vibrational energy that is converted to translational motion on a femtosecond timescale. Effectively, water molecules are driven into the gas phase and leave the tissue carrying along cells and proteins. The different timescale between laser pulses and the molecular relaxation processes that convert vibrational to kinetic energy avoids multiphoton ionization and the fragmentation of constituent proteins. Ren et al [1] based their research on the earlier demonstration by some of the co-authors that PIRL DIVE could rapidly extract small amounts of biological samples that could be analyzed without the need of additional processing, such as homogenization, enzymatic digestion, or the use of extraction buffers [14]. The enzymes collected by PIRL DIVE retain their structure and their activity [14], but what is even more impressive is that in their current work [1] the authors show that single proteins, viruses and cells collected from the ablation plume are both morphologically and functionally identical to controls. Specifically, the authors examined ferritin molecules, recombinant green fluorescence protein (rGFP), S. cerivisiae cells, and Tobacco mosaic virus (TMV) [1]. After collection from the ablation plume, ferritin maintained its characteristic toroidal structure and rGFP fluorescence was unchanged. Some S. cerevisiae exhibited damaged membranes but a significant number were undamaged and rapidly formed a colony. Most interestingly, the authors extracted TMV from inoculated tobacco leaves and were able to identify it by transmission electron microscopy. Furthermore, their data suggest that the extracted TMV retained its full functionality as determined by its capacity to infect additional tobacco leaves.

The next challenge for PIRL DIVE would be to extract intact cells from native tissues maintaining their viability. Furthermore, although the extraction capability of proteins from tissues has been demonstrated [14], the limits of PIRL DIVE spatial resolution mapping still need to be assessed. In principle, the dimensions of the laser spot should be the limiting factor and the molecules in the ablation plume from each tissue pixel could be separately analyzed by mass spectroscopy. This would enable researchers to monitor, for example, the transition between native and repair (fibrous) cartilage, between pristine blood vessel tissue and atherosclerotic plaques, and between skin tumor and the adjacent skin (figure 1).

Over the years, a number of different approaches have been proposed to minimize invasiveness while analyzing the surface of biological tissues, such as quantitative multiphoton microscopy [15] or in situ AFM analysis [16]. These techniques, along with many others, have evidenced how local changes in small clusters of cells and in their immediate environment are central in disease initiation and progression in pathologies ranging from cancer [17] to osteoarthritis [16, 18]. However, most current approaches still present limitations in terms of either their resolving power or their immediate clinical applicability. In fact, the capacity of rapidly mapping with high resolution and minimal invasion of local tissues in vivo could have far reaching consequences in understanding disease processes and most importantly in improving diagnostics. In this respect, PIRL DIVE could be especially relevant when considering that the local structural properties of the extracellular matrix play a key role in tumor invasion and aggression [17].

In this context, PIRL DIVE has the potential to be a game changer. Once implemented in a clinical instrument, potentially even coupled with appropriate
analytical devices, it would offer high resolution spatial mapping capacity and minimal invasiveness. In the future, this technique could provide the ability to identify early changes at the cellular level and in the local extracellular matrix, well before their effects become evident macroscopically.

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Figure 1. Schematic representation of the application of PIRL DIVE for high resolution sampling of healthy or diseased biological tissues.
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