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# X-ray phase contrast tomography with an immuno-histochemical approach

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**Summary.** — In the study of neurodegenerative diseases, a crucial point is the possibility of monitoring cells and tissues of interest within the body and tracking their modifications during the course of the disease or treatment. Currently, it is not possible to achieve this goal in-vivo, due to unsatisfactory image resolution, while conventional post-mortem analysis necessitates the use of sample-destructive techniques. In this work, we propose to exploit the special characteristics of nanoparticles and nanobodies to enrich the abilities of nano and micro-XPCT, an innovative technique already used in the study of neurodegenerative disorders, and enhance it to tissue-selective 3D imaging, in addition to the morphological information provided by the 3D tomography.

## 1. – Introduction

Neurodegenerative diseases are a heterogeneous group of disorders characterized by the progressive degeneration of the structure and function of the central and/or the peripheral nervous system, including neuronal loss in different anatomical and functional systems [1]. Neurodegenerative diseases affect millions of people worldwide; the risk of being affected by a neurodegenerative disease increases dramatically with age. This situation creates a critical need to improve our understanding of their etiology, and thus to develop new strategies for their treatment and/or prevention. However, thanks to the

 

recent research progresses, the development of treatments able to modify the neurodegenerative process has become possible for the first time; this has stimulated intensive pre-clinical research programs in animal models around the world [2]. Critical unmet needs in pre-clinical neurodegenerative disease research include the possibility of detecting and investigating specific neuropathological signatures as well as of following up the effects of therapies at the submicron scale. Ideally, this would require the availability of 3D maps of the whole brain in its natural status, rather than only partial volumes of excised post-mortem central nervous system (CNS) tissues, prepared for histology by blood removal, freezing at extremely low temperatures, manipulation with aggressive fixatives and various detergents. Direct 3D imaging would allow for the simultaneous analyses of i) alterations of various cell populations (neuronal loss, gliosis, peripheral infiltrates) in the brain; ii) structural changes in terms of cell density and organization (brain atrophy); iii) modifications of vascular networks and architecture integrity (cerebral angiopathies) [3]. Additionally, the ambitious challenge to achieve a 3D imaging of the highly complex neuronal and vascular networks communicating with disease-relevant cells, while preserving tissue chemistry, cannot be by using conventional immunohistochemical techniques, as these necessitate chemical tissue manipulation to add fluorescence/chromogenic reporters. Magnetic resonance imaging is a non-invasive imaging technique that can partially circumvent these limitations; however, in preclinical studies, it can only provide images with spatial resolutions as high as several tens of microns, which are not suitable for viewing individual neuronal cells or capillaries. Conventional X-ray tomography can instead provide submicron resolution images; however, it fails in producing high-contrast images when applied to soft tissues because of the very similar absorption coefficient of X-rays shown by the different CNS tissues components.

X-ray phase contrast tomography (XPCT) is a novel non-destructive imaging technique, which has shown a great potential in filling in the gap described before. XPCT allows performing multi-scale 3D biomedical imaging of neuronal and vascular networks with fields of views ranging from a single cell to the whole brain, without the need of slicing or pre-processing tissues; it therefore allows obtaining a 3D "virtual histology" of the samples [4, 5]. XPCT has appeared particularly useful in analysing low-absorbing bio-medical samples, with a gain in contrast resolution reaching up to 1000 folds that of conventional tomography [6]. For all these properties, XPCT has become the reference in the laboratory investigations of neurodegenerative diseases. However, XPCT has a limitation in that it cannot uniquely identify the cells of interest, but can only speculate on their nature from a morphological point of view [7].

## 2. – Metal nanoparticles

Metal nanoparticles (NPs) show X-ray-attenuation properties that make them suitable as effective long-circulating and biocompatible contrast agents for X-ray imaging and standard computed tomography [8]. Thanks to their unique features, such as size, high surface/volume ratio, surface chemistry and blood-brain-barrier (BBB) crossing propensity [9], some nanoparticles can be manipulated to bind selected biomolecules and for molecular probes.

## 3. – Nanobodies

Nanobodies (NB) are single-domain antibodies composed of the single heavy chain of antibodies that are expressed uniquely in camelids and lack the light chain. Unlike

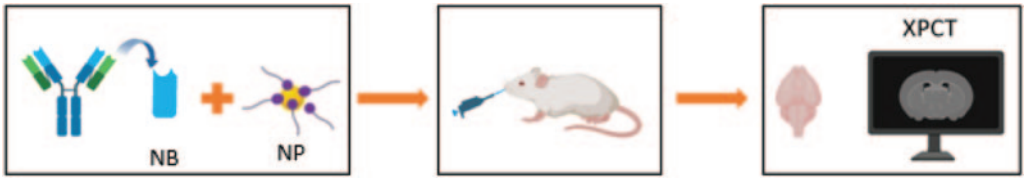


Fig. 1. – X-ray phase contrast tomography with an immuno-histochemical approach. Single domain antibodies conjugated with metal nanoparticles are administrated to mice and the brain is measured with an X-ray phase contrast tomography experiment.

classic antibodies (Ab), nanobodies can cross the BBB due to their size (about 15 kDa, 10 times smaller than a full Ab) and to the possibility of being genetically engineered to present a basic isoelectric point which increases their BBB-crossing propensity [10]. These properties also enable them to target antigenic epitopes at locations not easily accessible to conventional antibodies [11]. Such features, together with their stability and rapid clearance from blood, make sdAbs optimal candidates for molecular imaging in brain [12].

## 4. – Conclusions

X-ray phase contrast tomography is a powerful and useful technique in the biological field, in particular for the study of neurodegenerative diseases, allowing the 3D visualization of the vascular and neuronal networks, the surrounding tissue and the cells of interest. At now, the univocal identification of the cells is not possible, and this is the crucial limit of XPCT. The idea we introduce in this work is to combine the XPCT with a new complex composed of metal nanoparticles and nanobodies, in order to target specific cells of interest and detect them in the volume (fig. 1). This appears to be of great interest in the context of preclinical studies on neurodegenerative diseases, in which it is essential to detect and investigate specific neuropathological signatures and to follow the effects of therapies at the cellular level.

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