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press kit

# Titan Krios™ gives the Institut Pasteur a closer look at life

The researcher Jean-Claude Manuguerra.
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**The Institut Pasteur's new Titan Krios™ (Thermo Scientific™ Krios™ Cryo-TEM), from Thermo Fisher Scientific is an industry-leading electron microscope with advanced capabilities. It was officially unveiled today, July 12, 2018. With the Titan Krios™, biological structures and phenomena such as viruses, cell components and protein complexes can be visualized with an unprecedented level of detail. The Titan Krios™ microscopy system facilitates ultra-high-resolution observation of the most fragile samples in near-natural conditions, thanks to the use of cryogenic techniques. When linked with a highly sophisticated camera system, the imaging under cryogenic conditions provides outstanding three dimensional imaging possibilities with new levels of precision. By acquiring Titan Krios™, the Institut Pasteur is offering its scientists an extraordinarily powerful tool to observe cells and get a closer look at life.**

The Titan Krios™ system (Thermo Scientific™ Krios™ Cryo-TEM) installed at the Institut Pasteur stands 4 meters tall. It is one of the largest and most powerful microscopes in the world in an urban area. To house the microscope and provide optimal usage conditions, a new building was needed that would fulfill a number of requirements with regard to electromagnetic fields, temperature, humidity, vibrations and nitrogen flow.

The location and configuration of the Nocard building were meticulously planned: it has its own ventilation system to guarantee a stable environment; and the interior walls are armored to avoid any magnetic fields from outside the building (from the metro, cell phones, WiFi, etc.).

Olivier Schwartz, Scientific Director at the Institut Pasteur, says: "*The arrival of this remarkable microscope is a major scientific event for the Institut Pasteur's teams. We are all eager to discover the capabilities of the Titan Krios™ and to deepen our knowledge, and more broadly that of the scientific and medical community, through the data and images generated.*"

The Institut Pasteur was determined to overcome the huge challenges involved in installing this microscope in an urban environment so that it could help advance research in areas such as neurodegenerative diseases, pathogens and cancer.

"*Cryo-EM has changed the way scientists learn about neurodegenerative diseases, pathogens and cancers because of its ability to provide them with an unprecedented look at the structures of proteins and provide new insights in disease mechanisms," s*aid Mike Shafer, President, Materials and Structural Analysis, Thermo Fisher. *"We applaud the Institut Pasteur for being at the forefront of innovation, and their installation of our* Titan Krios™ (*Krios™ Cryo-TEM) is further proof of their commitment to advancing important research in these and other disciplines.*"

**TITAN KRIOS™: WHAT MAKES IT SO SPECIAL?**

The Titan Krios™ is fitted with a highly sophisticated camera that directly detects the stream of electrons that it fires, enabling scientists to visualize samples at an atomic scale (a tenth of a nanometer). This scale reveals countless details that would be invisible using other traditional research instruments. Scientists are therefore able to characterize the structure of protein complexes and also study their interactions in their natural environment.

> **A sense of scale**
>
> **With the naked eye,** humans can make out a detail measuring roughly 1mm from a distance of 3 meters.
>
> **Titan Krios**™ enables today's scientists to magnify an image millions of times (compared with magnification of just 10x with the first microscopes).

This knowledge will enable a vital contribution to the scientific community's understanding of the molecular mechanisms involved in diseases and the development of targeted treatments. More broadly, the images revealed by this microscope will be useful for specialists in a host of different disciplines including immunology, cell biology, bacteriology, virology, parasitology and neuroscience.


Titan Krios™ (Krios™ Cryo-TEM): This ultra-high-resolution "cryo-electron microscope", which resembles a 4-meter-high cabinet, is connected to computers that analyze and reproduce the images. Here, at the Institut Pasteur, in the new Nocard building.

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Designed by Thermo Fisher Scientific, the first Titan Krios™ (Thermo Scientific™ Krios™ Cryo-TEM) was produced in February 2008. It was developed in collaboration with the Max Planck Institute of Biochemistry in Germany. Recent advancements made to the design and imaging hardware, along with enhanced image processing and automation in the Titan Krios™, have helped to catapult the Cryo-TEM technique's success, which was awarded the Nobel Prize in Chemistry in 2017.

Cryo-TEM is a technique where a sample is cooled to cryogenic temperatures (below -180 °C), providing scientists the opportunity to create 3D images of proteins and viruses that couldn't otherwise be obtained. Understanding the structure of viruses and proteins helps determine how they function, potentially leading to prevention or treatment of the diseases they cause. For example, if you know how the HIV virus enters and infects a cell or how, with neurodegenerative disease, a tau protein aggregates and forms plaque in the brain, it makes it easier to design a drug for prevention.

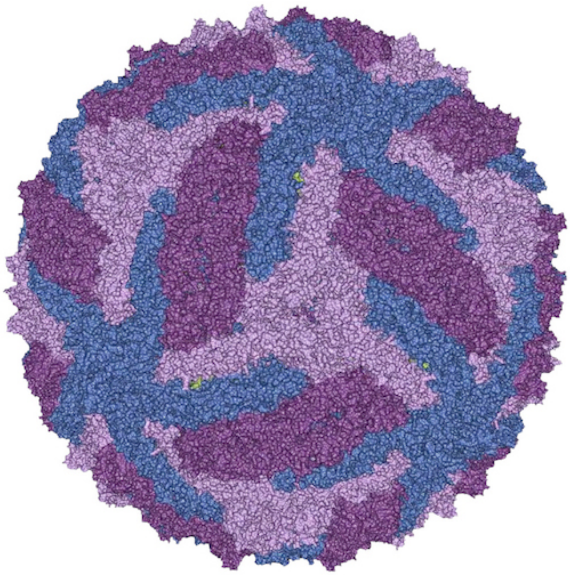
## PRESERVING THE NATURAL ENVIRONMENT WITH CRYOGENICS

The Titan Krios™ system has a valuable asset for studying samples in optimal observation conditions, thereby slowing down their denaturation: the sample is flash frozen at a temperature of -180°C to preserve them in their natural environment.

The microscope draws on the technique of cryogenic transmission electron microscopy (cryo-TEM) that emerged in the mid-1980s. Cryo-TEM involves rapidly freezing a biological sample to trap it in a thin layer of amorphous ice, then observing it using a suitable electron microscope. The frozen sample is kept at a very low temperature within the electron microscope, and images are recorded with extremely low levels of electrons in order to prevent damage to the sample.

Previously, determining the structure of a molecule involved isolating it then crystallizing it using a process that could be very lengthy, and which could often result in the denaturation of proteins that were too fragile or unstable. Specifically, this technique was not adapted to the analysis of molecular complexes located in cell walls, which are very important for therapeutic development. Nowadays, with the Titan Krios™ system, scientists talk about Single Particle Analysis (SPA) prepared by "cryogenization" or "vitrification", which allows to obtain a structure without having to crystallize the complex of interest.

The microscope helps to maintain the stability of samples under study, and its direct electron detector camera enables the automatic acquisition of thousands of images. By a high-speed recording of successive images, several frames can be aligned with respect to each other to increase the sharpness of the final image of proteins in their cellular environment: this refers to the ”movie” mode which revolutionized the Cryo-TEM, which benefits the Titan Krios™ system.



An image of the Zika virus, reconstructed in 3D. One of the advantages of the Titan Krios™ (Krios™ Cryo-TEM) is the possibility of long periods of data acquisition (two to five days on average, for reconstruction from large series of images). By using correlation techniques, these acquisitions can provide a 3D image.

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**THE MICROSCOPIC WORLD IN THE DIGITAL ERA**

The Titan Krios™ system can save scientists a considerable amount of time and enable them to examine highly fragile samples within their natural cellular environment. The microscope is also fully digital and automated: scientists can insert several samples (up to 12) simultaneously, then observe them successively in a much shorter period of time than previous generations of microscopes and generate a collection of images in record time, essential to the understanding of their research work.

By producing such huge swathes of data, this groundbreaking microscope opens up new avenues for research, but storing the vast quantities of information collected represents a major challenge. The Institut Pasteur will therefore be installing state-of-the-art IT equipment so that the data can be processed and stored properly.

As Michael Nilges, head of the Structural Bioinformatics Unit and Director of the Structural Biology and Chemistry Department at the Institut Pasteur, explains, "*The Titan Krios™ generates between half a terabyte and one terabyte of data every single day, which adds up to virtually 400 TB each year. The challenge is finding a way to store these reams of data so that any specialist in France can consult this amazing visual library, which I hope will enable scientists to discover new cellular and molecular processes and find out more about a part of the living world that has thus far remained unexplored.*"

## EXPERT TEAMS WORKING WITH THE TITAN KRIOS™ SYSTEM

Installing and working with such a powerful microscope requires highly specific skills. A dedicated new research unit has therefore been set up, directed by Prof. Dorit Hanein, a researcher in bioinformatics and structural biology at Sanford Burnham Prebys Medical Discovery Institute in San Diego. As one of the world's leading Titan Krios™ experts, Prof. Hanein also supervised the installation of the microscope at the Institut Pasteur.

She explains that: "*Optimization of the Titan Krios™ performance would harness its powerful imaging capabilities to analyze the various samples of investigators at the Institut Pasteur. Taking advantage of the Titan Krios™, my unit will continue developing the cutting-edge technological platform for quantitative integration of scales between macroscopic cellular behavior and high-resolution structural change. This workflow is central to my research aimed at understanding processes that cause disease at all scales by addressing a critical question in cell biology; how do cells employ large, molecular machines in cellular processes.*"

The new unit's research will focus on elucidating the basic processes of life, such as protein assemblies and cell signaling. Many pathogens disrupt these workings for their own survival and to invade host organisms and spread there. Identifying the basic mechanisms that underpin the organization of living systems will therefore have a major impact on research into a variety of biological processes and infectious strategies.

Several other Institut Pasteur teams will also benefit from this new equipment; potential uses include examining host cell integration and analyzing mechanisms associated with the emergence of neurodegenerative diseases and cancer.

## SCIENCE GETS A CLOSER LOOK AT LIFE

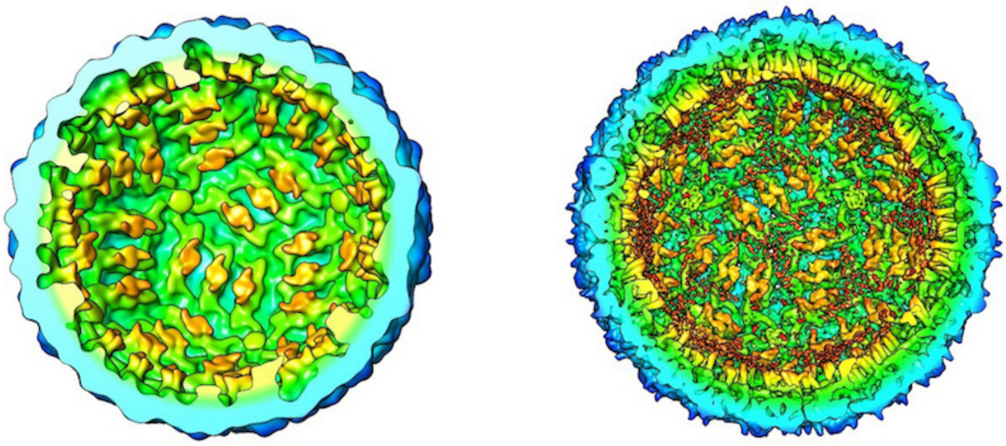
"*The microscopic world is amazing!*" says Olivier Schwartz, Scientific Director at the Institut Pasteur. "*The images obtained with microscopes can help to illustrate or even fuel our discoveries. In my research on the interactions between viruses and cells in the body, I have always used different microscopy techniques, and I have witnessed the incredible progress made over the last few years, whether in optical microscopy, which is best for observing cells, or electron microscopy, which is mainly used for visualizing viruses.*"

The scientist continues: "*Now we can combine both optical and electron microscopy, a bit like Google Maps: we can get an overall view, observing infected and non-infected cells, for example, then look at the finer details, right down to viral particles on a cell membrane.*"

The association of different microscopic methods developing a lot in the world, is called "Integrative Microscopy", and puts in interaction several disciplines (virology, cell biology etc.) which complete more and more their research with the help of Cryo-TEM. The addition of the Titan Krios™ (Krios™ Cryo-TEM) to the Institut Pasteur's arsenal of technologies will thus strengthen collaborations in the main branches of life sciences for the benefit of patients.

The Titan Krios™ system will enable scientists to observe cells in their natural environment. A metaphor from Professor Dorit Hanein provides an effective illustration for this phenomenon.

According to the scientist, "*The Titan offers a robust technology through computational analysis to get a closer look at life. Take the example of a swimmer: to understand "why" an athlete swims, one must observe how each part of this machine works, how these parts work together and to "see" how it's working in the overcrowded ocean. Observing with high precision how parts work in isolation might be limiting, as the swimmer's movements are also dictated by its interactions with its neighbors. Hence the Titan Krios™ allows us to observe living organisms within their own neighborhood and universe, thus elucidating networks and behaviors that are fundamental, in the true sense of the word.*"



Left: The dengue virus seen under a traditional electron microscope.

Right: The dengue virus observed using the Titan Krios™ (Krios™ Cryo-TEM) microscope.



**A LARGE-SCALE PROJECT**

Since 2014, the Institut Pasteur has embarked on a number of innovative projects designed to promote synergies and advance scientific research. Providing scientists with the means of observing cells in their natural environment to shed light on the molecular processes involved in disease emergence was one of these ambitious projects (the "Nano imaging initiative"), launched back in 2015.

The availability of one of the most powerful microscopes in the world in the center of Paris is a symbol of excellence and scientific competitiveness in the field of structural biology for all Institut Pasteur researchers, as well as Institut Pasteur partners. Several teams working in immunology, bacteriology, virology, parasitology and neuroscience will now be able to extend their research.

The installation of this ultra-high-resolution microscope on the Institut Pasteur's Paris campus is a key part of the CACSICE (Center for the Analysis of Complex Systems in Complex Environments) project, led by a consortium composed of the Institut Pasteur, the CNRS and Paris Descartes University and coordinated by Michael Nilges.

In 2011, the CACSICE project was awarded an "Equipex" ("Equipment of Excellence") grant of €7.5 million by the French National Research Agency (ANR) in connection with the French government's Investing in the Future Programs, to help fund the acquisition of the microscope.

Arrival of the microscope on the Institut Pasteur Paris campus in December 2017, on the left. The Nocard building, on the right.
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The installation of the Titan Krios™ (Krios™ Cryo-TEM) microscope at the Institut Pasteur required a major investment of more than €10 million overall (for the purchase of the microscope and camera and the building of the new Nocard facility). A lengthy crowdfunding campaign was launched in 2016.

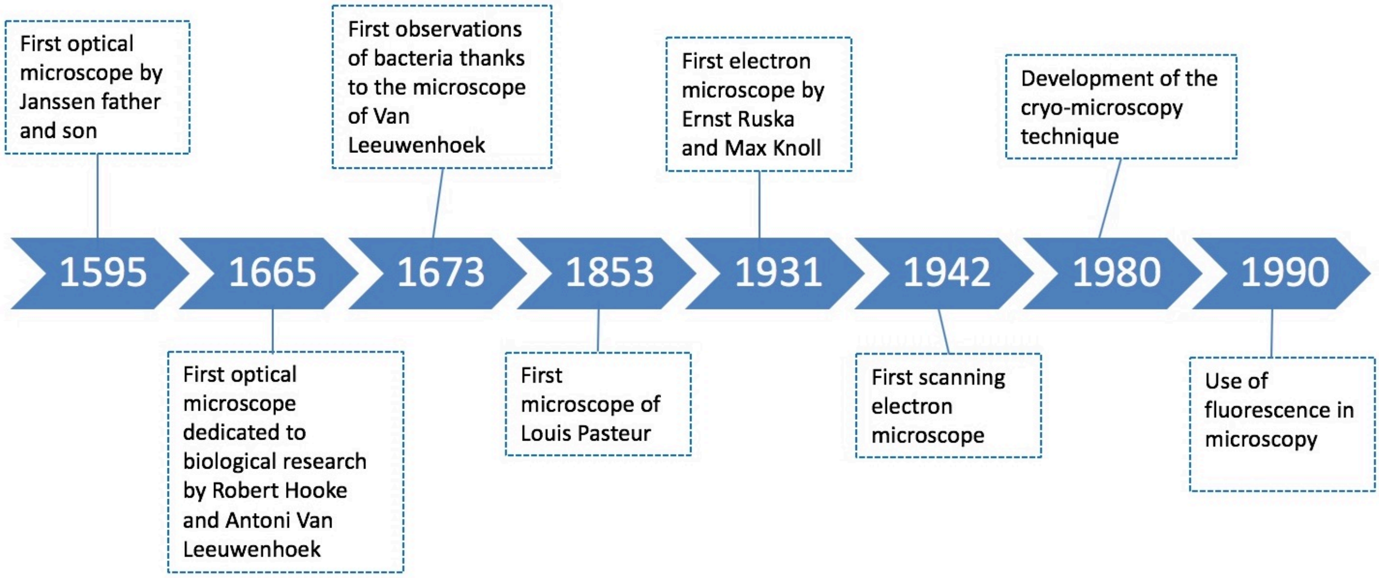
Dr. Jean-François Chambon, Vice-President Communications and Fundraising, explains: "*We approached our sponsors and major donors, and a huge number of individuals also got involved in this fundraising campaign. Their response helped us to raise more than €1.5 million. It was this show of generosity that enabled the Institut Pasteur to finalize the project and to provide our scientists with this new equipment.*"

For the Institut Pasteur, which is celebrating its 130th anniversary this year, the acquisition of the Titan Krios™ microscope, its installation in a dedicated facility and the recruitment of experts specializing in cryo-electron microscopy demonstrate its ambition to remain at the forefront of innovation for health.

**MICROSCOPY OVER TIME**

Microscopy has developed in several stages, many of which have had a direct impact on the research carried out at the Institut Pasteur.

Here's a look back at some of the major milestones:




Optical microscopy

**1667:** Robert Hooke, one of the early pioneers of microscopy, writes the book Micrographia, the first treatise on the subject.

**1673:** Antonie Van Leeuwenhoek is the first scientist to observe bacteria, which he names "animalcules".

**1853:** Louis Pasteur acquires his first microscope.

**1882:** German scientist Robert Koch discovers the tuberculosis bacillus using a microscope.

**1889:** The Institut Pasteur inaugurates its photomicrography laboratory.

**1894:** Institut Pasteur scientist Alexandre Yersin discovers the pathogenic bacteria responsible for plague.

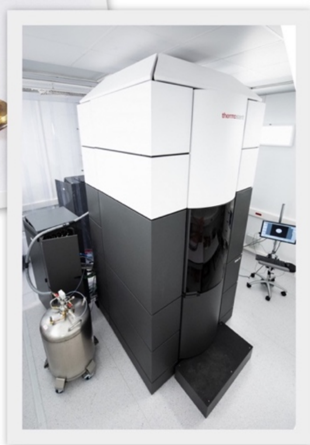
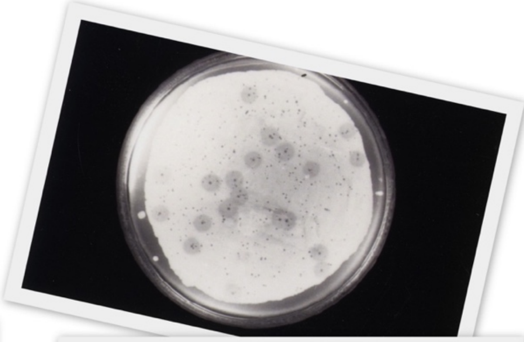
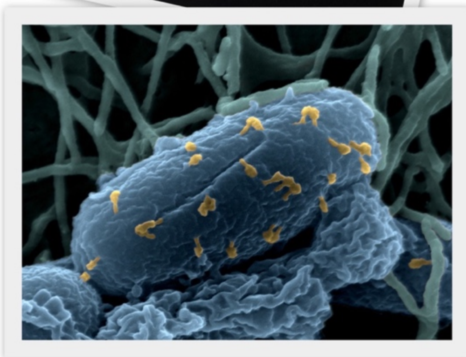
Electron microscopy

**1930s:** German engineers create the first prototypes of electron microscopes. Instead of using light (made up of photons) and glass lenses, they come up with the idea of using electrons, accelerated in vacuum through cathode ray tubes, and electromagnetic lenses.

**1942:** The Virus Department acquires the first electron microscope at the Institut Pasteur.

**1963:** At the same time as his counterparts in other countries, Institut Pasteur scientist Charles Dauguet produces the first images of the rabies virus in France.

**1983:** Charles Dauguet produces the first microscope image to illustrate the discovery of HIV.

Left: Louis Pasteur's first microscope (top); the Titan Krios™ (Krios™ Cryo-TEM) microscope (bottom).

Right: Photo of an experiment on bacteriophages carried out by Félix d'Hérelle (1936-1940, top); high-resolution image of bacteriophages on the *E. coli* bacterium (2011, bottom).

Fluorescence microscopy

**1962:** Discovery of GFP (green fluorescent protein), a protein from jellyfish that acts as a marker for viruses, bacteria and cells.

**1974:** A Central Electron Microscopy Station is set up at the Institut Pasteur.

**2001:** A Dynamic Imaging Platform is set up.

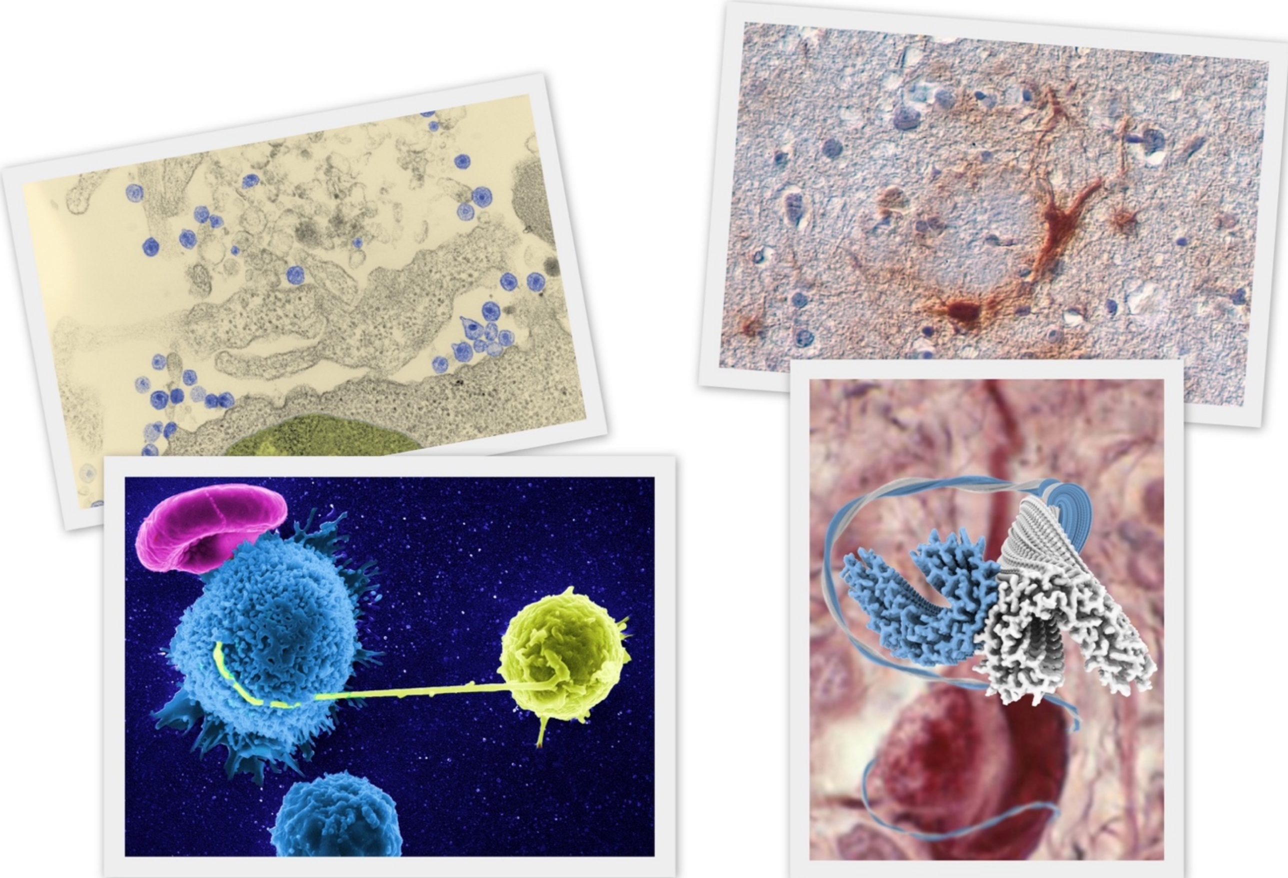
Cryo-microscopy

**1980s-90s:** Three scientists, Jacques Dubochet, Joachim Franck and Richard Henderson, who were awarded the Nobel Prize in 2017, carry out complementary research that contributes to the development of the cryo-electron microscopy technique. In 1990, Richard Henderson obtains the first 3D image of a protein.

**2000:** acquisition of a first cryo-microscope by the Institut Pasteur.

**2009:** the first team of cryo-microscopy experts is created at the Institut Pasteur.

**2018:** A Titan Krios™ (Krios™ Cryo-TEM) microscope, which uses the technique of cryo-electron microscopy, is installed in the Nocard building at the Institut Pasteur.



Left: First microscope image illustrating the discovery of HIV, produced by Charles Dauguet (1983, top); HIV-infected lymphocytes observed by scanning electron microscopy, images produced by Olivier Schwartz's team (2009, bottom).

Right: Amyloid plaque characteristic of Alzheimer's disease (2009, top); image produced by the Titan Krios™ (Krios™ Cryo-TEM) of a paired helical filament associated with Alzheimer's disease (© Sjors Scheres, bottom).

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