

“Nowhere near the End of the Possibilities”

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Heidi Cartwright, *Circuit Collage*, Prince of Wales Medical Research Institute, Randwick, Australia, 2000



Optical technologies are essential to medicine and biomedical research. In the past, they have made valuable contributions to clinical diagnostics and in decoding biological processes. This led to the development of numerous endoscopic and microscopic techniques that provide important information on the internal workings of the body and permit research into the biological processes of cells or tissue samples with high

spatial resolution at a cellular or sub-cellular level.

Intensive Research and Development. In recent years, researchers have increasingly succeeded in displaying biological processes non-invasively at the cellular/molecular level using optical methods. The term molecular imaging is being increasingly used to describe several partially competitive imaging technologies that enable the visualization of biological processes in intact organisms.

In the future, this will lead to earlier and more exact diagnoses, as well as more specific therapies for diseases. However, this requires intensive research and development which is also the objective of the molecular imaging innovation alliance launched in October 2007 in Berlin.

The mouse model. Let us take an example of what we are working on at the Center for Stroke Research in Berlin (CSB) at the Charité: how can you non-invasively display inflammatory processes in the brain following a stroke? We are first working on a mouse model for this purpose. Initially, highly specific marker substances must be developed to visualize inflammatory processes in the brain. The first step is to identify suitable molecular targets that play a key role in inflammatory processes. The second step involves developing molecules (ligands) that very precisely bond to the identified targets. In the third step, these ligands are marked for imaging, e.g. with radioisotopes for nuclear medicine or fluorescence dyes for optical imaging.

Optical methods provide enormous advantages in the development of such marker substances. However, they are not only ideal tools for the evaluation of targets and ligands in model systems, but are also suitable for use on patients as long as the target tissue is not too deep in the body. This is where optical procedures reach their limitations. On the other hand, optical methods are known for their high level of sensitivity: very minute traces of a fluorescence dye can be detected. As a result of the wide va-

riety of microscope techniques, optical methods offer the possibility of examining the distribution of a substance non-invasively and also with high spatial resolution at the cellular or sub-cellular level. Thus, stroke-induced inflammatory processes can be examined non-invasively at the cellular level following the injection of a fluorescently labeled inflammatory marker and after removal of tissue samples (ex-vivo).

Deep into the brain. An example of this is white light and fluorescence images of a mouse with a stroke in the left hemisphere (see page 29). We injected the mouse with a fluorescence dye with which the stroke-induced inflammatory processes can be imaged. The high fluorescence intensity above the hemisphere affected by the stroke (see page 29, fluorescence image of the head of the mouse, lower left) can be seen in the living mouse even deep into the brain and through the cranium. This image was recorded in only a few seconds.

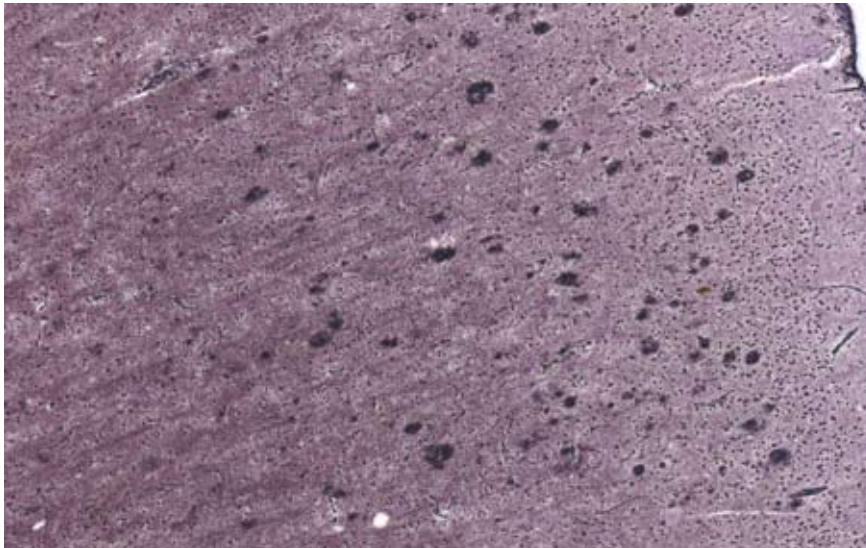
The removal of tissue samples shows that the fluorescence dye actually images the inflammatory processes in the brain. We succeeded in verifying the marker in the tissue affected by the stroke (see page 29, brain section; the color of the stroke tissue is clearly less intense than living tissue after staining). Furthermore, using a microscope, we were able to verify the specificity of the marker at the cellular level for certain inflammatory cells after preparing thin tissue sections and specific cell staining. The yellow-edged cell is an inflammatory cell that has bonded with the marker

The person

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Original preparation by Alois Alzheimer, 1907, presenile dementia, cortex of the brain, Bielschowsky's silver impregnation. MIRAX digital slide scanner.

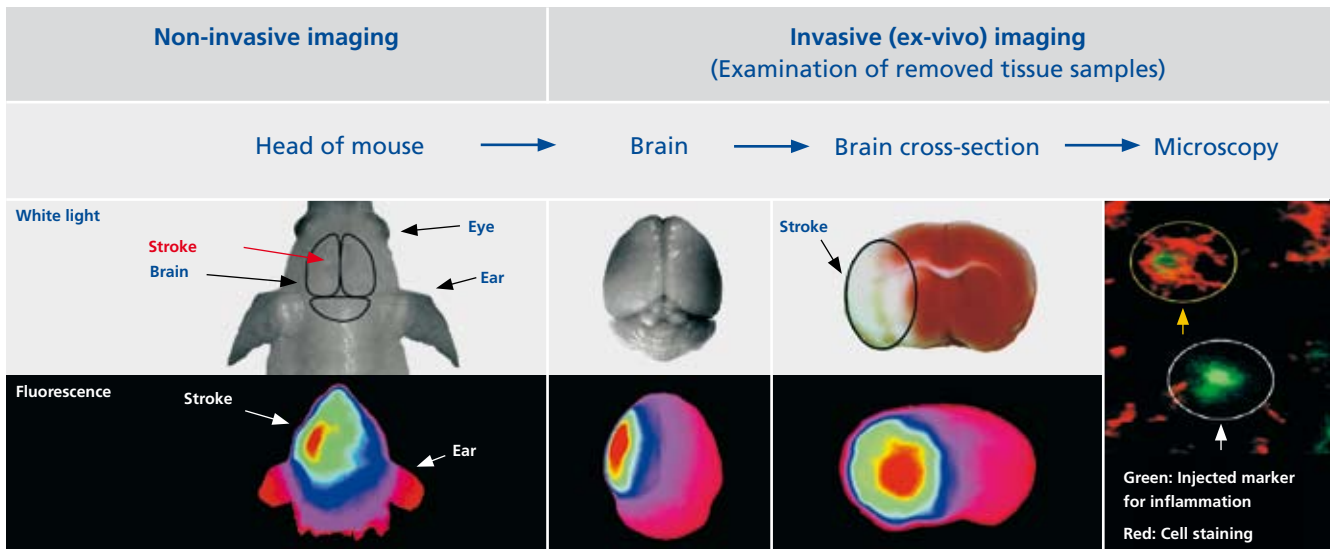
and that belongs to the stained sub-type. The white-edged cell is also an inflammatory cell, which belongs to another sub-type.

Marking and examining. Optical technologies are not only relatively easy, affordable and fast, they also offer the possibility of examining several biological processes simultaneously. Additionally, the different marker substances are stained with different fluorescence dyes and detected separately (multi-channel imaging). This technique can also be used if marker substances are to be tested for their specificity: with a number of diseases such as tumors or inflammatory processes there is a malfunction of the blood-tissue barrier. This leads to an unspecific accumulation of sub-

stances in the affected tissue. Thus, a major part of the detected signal is attributable to unspecific accumulation. In order to estimate this portion, multi-channel imaging can be used to mark and simultaneously examine an unspecific marker substance and the specific marker substance with different fluorescence dyes.

The new filter systems. However, we are nowhere near the end of the possibilities offered by optical techniques. Improved fluorescence dyes that display less bleaching or have a higher fluorescence quantum yield can make the measurements even more sensitive. New filter systems that better separate the excitation light and the fluorescence light or enable improved simultaneous mea-

surement of several fluorescence dyes are also needed. Much will happen in the area of imaging systems in the future. This includes the development of new fluorescence tomography systems that deliver 3D and quantitative data sets and are adjusted to the requirements for use in model systems and the needs of patients. These examples show that optical technologies still have enormous development potential for the future.



White light and fluorescence images of a mouse with a stroke in the left hemisphere after injection of a fluorescence marker for the specific visualization of stroke-induced inflammatory processes.

As can be seen using the high fluorescence intensity above the hemisphere affected by the stroke (see fluorescence image of the head of the mouse, lower left), the fluorescence marker can even be non-invasively detected with high sensitivity deep in the brain through the cranium. This image was recorded in only a few seconds.

After removal of tissue samples, the marker was successfully verified in the tissue affected by the stroke (see brain section; the color of the stroke tissue is clearly less intense than living tissue after staining). Furthermore, using a microscope, it was possible to identify cell types that bind the marker and thus verify the specificity of the marker at the cellular level for certain inflammatory cells after the preparation of thin tissue sections and specific cell staining.

The cell with a yellow border is an inflammatory cell that has bonded with the marker and belongs to the stained sub-type. The cell with a white border is also an inflammatory cell that belongs to another sub-type.