DOI DataCite: https://doi.org/10.5281/zenodo.439036 S. Mottin Identifier: http://orcid.org/0000-0002-7088-4353

Reference BibTeX:
@article{2012_mottin_36,
TITLE = {Bird Brains : Using Picosecond Optical Tomography to Assess Neural Activity},
AUTHOR = { Mottin, St.phane and Montcel, Bruno },
JOURNAL = {Optics & Photonics News of Optical Society of America},
VOLUME = { 23},NUMBER = {10},
PAGES = {40-47},YEAR = {2012}, DOI = {10.5281/zenodo.439036}
KEYWORDS = {Brain;Hemodynamics;Optical imaging;Oxygen;Blood;Streak cameras;Optical tomography;Lasers;bird},
}

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Bird Brains

Using Picosecond Optical Tomography to Assess Neural Activity

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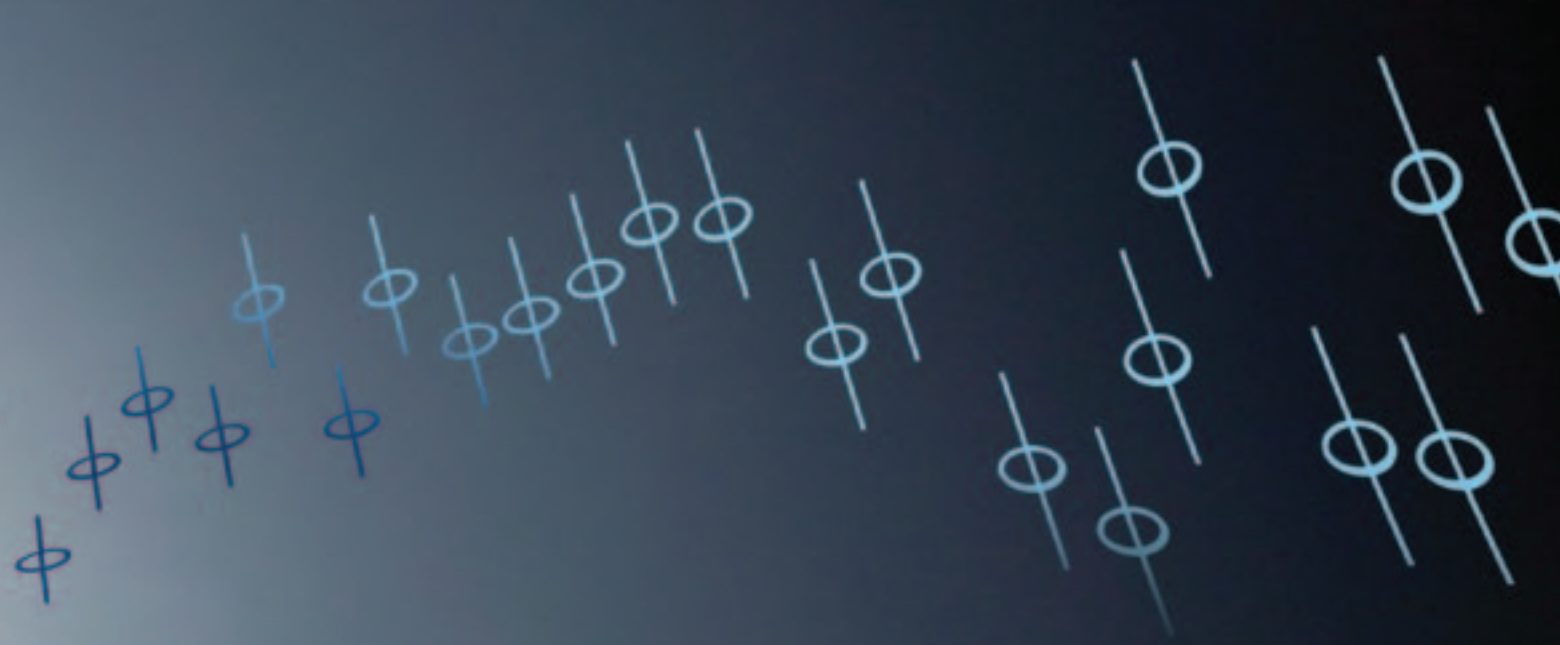

Imaging the brains of birds with picosecond optical tomography (POT) gives scientists a fascinating look into how these animals respond to calls and songs. This work establishes that POT can work with functional magnetic resonance imaging to produce a multimodal quantitative imaging technique that could one day be used in clinical medicine.

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Our thoughts, memories and actions are the product of the nerve cells in our brains working in harmony. Technologies that allow scientists to observe how our nerve cells function—or malfunction— can be used to diagnose a wide variety of neurological and vascular diseases, such as Alzheimer's and heart disease.

One way of assessing brain activity is by measuring blood flow and its relationship to nerve activation. An increase in the amount of oxygenated blood flowing to a region in the brain means that more nerve cells are firing. The reverse is also true. A low rate of blood flow indicates decreased brain activity because less oxygen is getting to the nerves. The term "brain respiration" refers to the relationship between oxygen consumption and nerve activity.

Current methods for measuring brain activity don't produce a lot of quantitative data on oxygen consumption. One of these methods—functional magnetic resonance imaging (fMRI)—has been used for some time to measure brain activity by detecting changes in blood flow. This method produces detailed pictures, but it cannot measure the rate of oxygen exchange in the blood. A new technique called picosecond optical tomography (POT) may fill in the gaps left by fMRI.

Birds and humans have similar forebrain structures—the part of the brain responsible for learning.

We used POT and fMRI techniques to measure brain respiration in zebra finches when they were engaged in an activity that stimulated their brains: listening to songs and calls. The imaging technique allowed us to calculate different levels of oxygen consumption in the brain when the birds heard songs vs. when they didn't. Hopefully this technique—or the findings derived from it—can one day be applied to humans, yielding valuable new insights into vascular and neurological diseases.

Why the zebra finch?

Birds and humans have similar forebrain structures—the part of the brain responsible for learning. Our metabolisms are also alike—we both expend large amounts of energy to maintain high and constant body temperatures. Like humans, zebra finches form social bonds with each other; they often share childrearing responsibilities; and they use listening and vocalizing with each other to learn how to communicate—all activities governed by the forebrain. Zebra finches also have a long life span that leads to an accumulation of learning experiences.

Little is known about the relationship between forebrain respiration and a bird's ability to learn and sing songs. To better understand how this part of the zebra finch brain uses oxygen—and to infer how similar processes may work in humans—we have designed a method for using *in vivo* POT imaging to measure the full time-course of oxygen transport during brain activity. POT will allow us to observe this reaction at previously unreached precision measurement with respect to reaction time and activity location. If we understand these processes in the zebra finch's brain, we are one step closer to understanding similar processes in humans.

Measuring brain activity

Our lab is using white laser POT to image brain respiration in zebra finches *in vivo* while they listen to familiar calls and songs. The area of the forebrain that controls singing in the zebra finch is made of clusters of nerve cells that are less than 1 mm^3 in size. Typically, researchers will examine these clusters by staining tissue samples post-mortem or by placing electrodes on the bird's scalp to measure nerve activity. However, neither of these techniques provides precise data for the amount and duration of brain activity while the bird listens to calls and songs.

In order to get a clear picture of brain respiration in the zebra finch, we need a reliable, quantitative method that allows real-time, non-invasive monitoring of brain activity with sub-millimeter resolution (that is, the ability

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Meet the Zebra Finch

Aside from being an excellent test subject for brain imaging studies, the zebra finch ...

- Is native to Australia, Indonesia and East Timor.
- Inhabits grasslands and forests that are close to water.
- Is a popular cage and aviary bird.
- Forms lifelong pair bonds; males serenade females in courtship.
- Grows to about 10 cm long.
- Prefers seed—especially spray millet.
- Has a lifespan of 5-10 years.
- Is a messy eater and boisterous singer.

to see areas smaller than the 1 mm^3 nerve cell clusters). A combination of POT and fMRI techniques fits the bill, but POT uses transmitted light to probe the auditory regions of the bird's forebrain and to produce the quantitative data we require.

Functional MRI can reveal blood flow changes in the brain through noninvasive high-resolution images, but it is difficult to quantitatively interpret fMRI results. The first drawback to this method is that brain tissue causes a lot of light scattering. The second is that an absorption coefficient must be applied for hemoglobin to account for the light it absorbs, and that constant is not exceedingly accurate when it is used in the visible part of the spectrum on the highly vascular membrane that encases the brain. Functional MRI is only accurate within a few hundred micrometers and a window of a few dozen seconds.

In order to quantitatively measure brain respiration, we need to be able to closely measure hemoglobin (Hb), which changes its molecular structure when it is carrying oxygen. That's where the use of near-infrared spectroscopy techniques such as POT comes in. Using POT, we can image cerebral tissue through an intact skin and skull because of the weak absorption of biological tissues in the near-infrared spectral window—in fact, absorption is conveniently localized to the Hb in red blood cells. During light propagation, the intensity decreases according to absorption and scattering. As a result, blood oxygenation levels can be deduced from the measurement of transmittance in turbid media (a diffuse optical method). POT can deduce activity at a submillimeter level and a window of picoseconds for time-of-flight (i.e., blood flow velocity) and a window of milliseconds for Hb concentrations.

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Our technique

In the near-infrared, optical imaging can provide two types of information. First, it measures the variations of light absorption. Second, it can quantify the variation of scattering, which relates to physiological characteristics that indicate an activated nerve—such as the swelling of nerve cells and blood vessels.

It has been a challenge to produce reliable optical measurements of oxygenated hemoglobin (HbO_2) and Hb concentrations in tissue. HbO_2 and Hb present very different absorption profiles—varying up to 650-700 nm. Some data can be obtained by using near-infrared lasers positioned at the scalp, but applications in humans and lab animals have been limited due to technical controversies about accuracy, anatomical precision and the scattering effects caused by the interaction among the skin, skull and brain membranes.

For our studies, we combined near-infrared spectroscopy (spectral-POT) and diffuse optical tomography with contact-free spatial imaging (spatial-POT) for imaging zebra finch brains *in vivo*. The patented spectral-POT is based on the variable behavior of the HbO_2 and Hb absorption spectra; this provides reliable molecular fingerprints for HbO_2 and Hb. Furthermore, the optical signals are integrated into a selected picosecond time-of-flight window that only probes the targeted deep brain structures.

This approach allowed us to quantify an evoked change in brain blood flow with submicromolar sensitivity and submillimeter spatial resolution. Moreover, the exact position of this imaged segment (5 mm X 0.2 mm) on the bird's head could be controlled using the naked eye by shining the intermediate slit with a He-Ne laser and adjusting the position of its image on the scalp at precise coordinates. In the case of such a small animal, this functional imaging system allows us to analyze the resolution limits of this class of transcranial optical method.

We used the laser source, a Ti:sapphire chirped pulse amplifier, to generate a 250-mW white-light supercontinuum. The spectral

shape was adapted to get a homogenized response at the photocathode of the camera. For *in vivo* measurements, we used only 10 µJ/mm^2 and one point of illumination. We don't need 250 mW in this application, but it could be useful for imaging a human brain or multiple sources on the scalp.

Our results

We have recorded the full time-courses of picosecond time-resolved transmittance measured by POT in zebra finches. To establish a calibrated functional method, we studied brain activity in the birds when they were listening to songs from other zebra finches. The spectrum peak was 760 nm, and the isobestic region (a specific wavelength at which two chemical species have the same molar absorptivity) of 800 nm was relatively stable despite the increase in noise induced by the laser pump wavelength (825 nm and 170 fs).

During the two seconds following the onset of the bird song, Hb and HbO_2 levels significantly decreased. Shortly after this decline, HbO_2 levels rose significantly until they reached a plateau. As one might expect, these changes were localized above the auditory forebrain areas of the birds. During the post-stimulus period, all areas showed a significant decrease in transmittance when compared to the rest period when no songs were played.

These results show that oxygen recoupling (Hb to HbO_2) was less localized than uncoupling (HbO_2 to Hb). The number of Hb and HbO_2 pulses was lower for the auditory areas of the brain than for more lateral positions in the brain, indicating that recoupling was faster in these areas.

We also imaged the sagittal midline, and no significant changes were observed during the activation period—which was expected. However, this result should be considered carefully because distinguishing arterial, capillary and venous compartments is not straightforward in diffuse optical imaging.

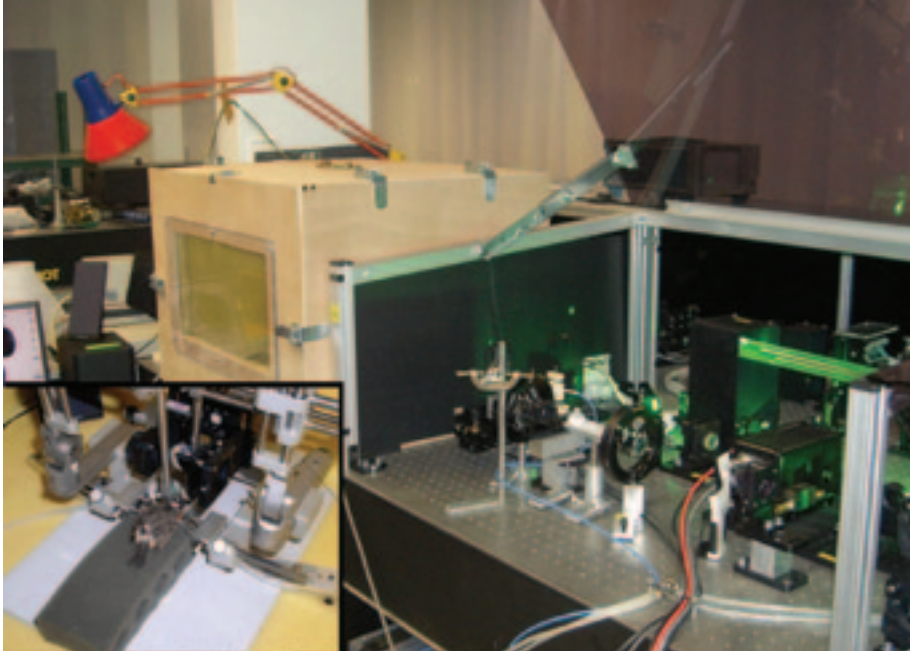
How we used POT and fMRI

As we noted earlier, POT and fMRI can complement one another very well, since the former can distinguish between HbO_2 and Hb, while the latter achieves submillimeter spatial resolution over the entire head. By choosing parameters similar to other published fMRI experiments, we sought to establish a correlation between the fMRI signal and changes in Hb and HbO_2.

The time courses of fMRI displayed a sharp rise and an overshoot when the bird first hears the song and an undershoot during the post-stimulus period. The decrease in Hb increased the magnetic resonance contrast and led to an increased fMRI signal. There is a direct link between the fMRI signal and decreasing levels of Hb in mammals and birds. However, the decreasing levels of Hb measured by POT exhibited a faster response to changes in stimulus, suggesting that the fMRI signal is a more convoluted response to hemodynamic changes.

Use in humans?

While it is not yet possible to use POT to measure brain activity in humans, it is worth pursuing since the benefits could be so great. There are a handful of limitations that make this technique out of reach for now. For example, when near-infrared light is sent into a human head, it must travel through at least 15 mm of tissue to reach the brain's surface—this is too deep for current POT technology. Furthermore, some anatomical structures prove to be obstacles for accurately measuring light scattering. For example, the gap between two of the membranes encapsulating the brain causes high and unpredictable scattering



Experimental setup: The picosecond optical tomography (POT) system includes a picosecond streak camera and a tunable Ti:Sapphire regenerative amplifier. (Inset) Anaesthetized zebra finches are precisely placed into a frame in order to probe the auditory regions of their forebrains.

Courtesy of Mottin and Montcel

because of the interference caused by strands of connective tissue between the membranes and cerebrospinal fluid.

Another structure that makes accurate scattering measurements in humans difficult is the innermost membrane surrounding the brain, which is called the pia mater. Its surface is highly vascularized, and the hemoglobin in these blood vessels interferes with measuring Hb levels of blood vessels in the actual brain tissue. Taking into account that light has to go through these structures twice to be detected non-invasively, it is not hard to see why POT is not an appropriate clinical tool at the moment. But recent progress in mathematics opens new perspectives in submillimiter resolution in humans.

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For the quantitative measurement of absorption into scattering media, three optical approaches have been developed: steady-state, frequency-domain and time-domain. The optical systems for these measurements are often complex, as are the algorithms for the inverse mathematical problem of recovering the absorption and scattering coefficients of other tissues. Moreover, depending on the number of chromophores (colorful molecules like Hb) to be measured, optical measurements are performed with one, two or several wavelengths. The use of spectroscopy (more than 100 wavelengths) also opens new perspectives for clinical applications of POT.

Using POT in the lab

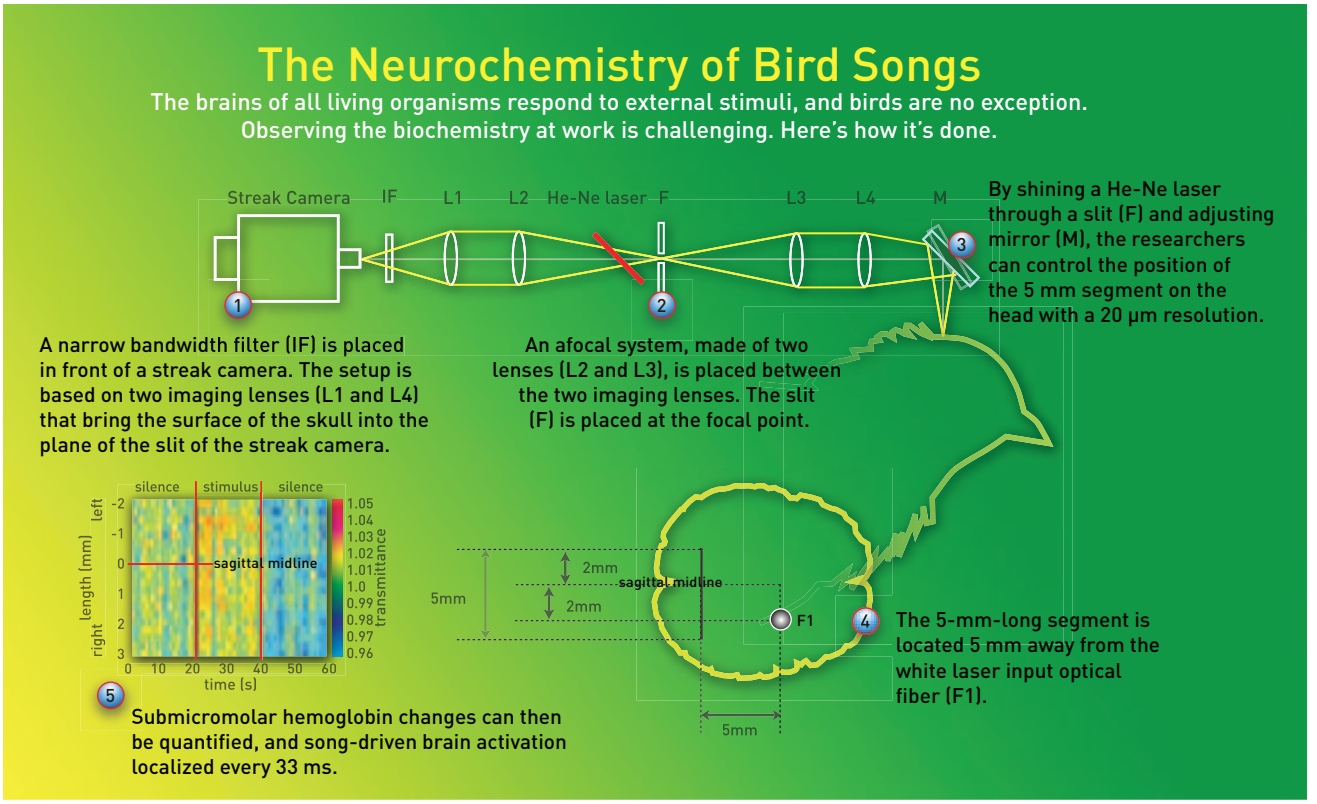
POT has proven to be a reliable experimental tool for characterizing the optical properties of diffusive media like brain tissue. It enables access to a whole distribution of photon times-of-flight, giving much more information on the optical and structural properties of the tested material. With in-depth scanning using POT, each photon time-of-flight corresponds to a specific homogenized exploration volume. It is therefore promising to combine POT with transverse imaging properties to get a complete picture of probed brain tissues.

Deep brain tissues can be monitored by POT with guide cannulas. In this case, POT could be combined with other optical methods such as optogenetics. It might also be used with time-resolved emission spectroscopy and imaging techniques. In all other *in vivo* optical methods, intrinsic absorption properties are always present, making POT a useful tool.

For 35 years, many optical tomography designs and processing algorithms have been developed, but none can reliably measure the absolute variation of absorption in the brain. The limitations are due to the organ's spatial heterogeneity, which is linked to the complex 3-D structure of the head—including the scalp, skull, cerebrospinal fluid, blood vessels and nerve cells—and to the tissue's molecular heterogeneity.

Compared to fMRI, POT instruments are relatively low-cost (typically less than $100,000) and portable. The picosecond time-domain seems to be the best choice for a robust quantitative method in such a complex organ. The spatial resolution of diffuse optical tomography techniques is several millimeters, but a lateral resolution of 250 µm could be obtained (for penetration depths of about 4-6 mm, distance between illumination-detection of 5 mm). With a refractive index around 1.4, typical time-of-flight is 5 ps for a depth of 1 mm. This does not correspond to the anatomical resolution limit, which is linked to the homogenization of scattering and absorption properties.

POT demonstrates the occurrence of strong reactivity in the brain blood vessels of the zebra finch, an animal with a long lifespan. Several studies indicate that age-related changes in vascular reactivity are important contributing factors to mild cognitive impairment in aging mammals. Also, the role of oxidative stress as a determinant of longevity is still open to question, and these results could thus shed light on this role.



Outlook

Functional MRI has transformed neuroscience and cognitive science. While the technology has not completely evolved, it is not brand new either. Rather, it is in its teenage years, with all the growing pains that accompany that age. If we can use POT to resolve the relationship between the fMRI signal and brain activity/blood flow dynamics, functional MRI may finally become a mature technology.

In recent studies with anesthetized rats, a functional ultrasound method has succeeded in imaging transient changes in blood dynamics in the whole brain. Because light and sound can influence each other through opto-acoustic and acousto-optic effects, this new technology is a method of choice for recording fast neurovascular events in combination with POT or similar approaches.

Other optical methods could also be used with POT, including chemometric luminescence lifetime imaging (measuring tissue O_2, pH, CO_2, etc.), quantitative electric activity imaging with voltage-sensitive dyes and immediate early gene expression imaging. We hope that someday POT can be used to measure brain activity and its relationship to blood flow dynamics in humans with the same spatial resolutions as our research with the zebra finch. OPN

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