## *General system scheme and definition of units*

The tACS-EEG closed-loop system incorporates an intermittent stimulation procedure with separated sliding windows for EEG acquisition and stimulation. Three modes of the phase relation between the alpha oscillations and tACS signals were integrated and studied in the experimental sessions: in-phase (directed on the minimal phase lag), anti-phase (directed on the phase lag close as possible to 180°), random phase (utilized for validation of in-phase and anti-phase stimulation effects). In order to satisfy the condition of real-time functioning and minimize optimization and transduction delays – the model with all data processing functions was implemented in C++ language (Qt environment). The general model scheme is presented on *Figure 2.1a*.

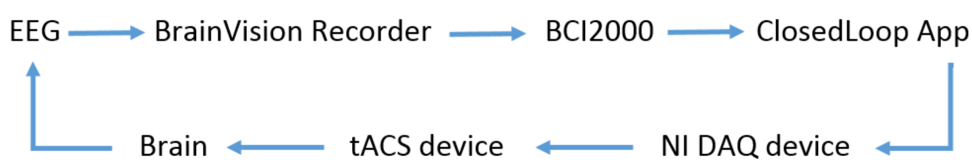


*Figure 2.1a General model scheme*

**EEG** is represented by BrainProducts amplifier and electrodes for recording brain activity, **BrainVision Recorder** – by an official software for the amplifier and its RDA-client for transmitting the data, **BCI2000** – by RDA signal source module and UDP streaming from brain-computer interface software [Schalk lab, 2004], **ClosedLoop App** – by the control application written in C++, **NI DAQ device** – by National Instrument USB 6229 device for generation of the stimulation signal and transmission of it to the stimulator input, **tACS device** – by neuroConn DC-STIMULATOR PLUS and 2 stimulation rubber electrodes (40x40 mm) attached to the brain.

## *Interactions between units of the closed-loop system*

The following paragraphs provide a detailed description of the implemented procedures for interactions and data transmission between different units of the developed system. As it is visible from the general model scheme (*Figure 2.1a*), the presented system has a linear closed-loop unidirectional architecture consisting of 7 components and 7 connections between them. Importantly, for precise execution of control commands and efficient signal processing, all of these connections (except **Brain to EEG**) require either a particular configuration by a set of available parameters and functions for utilized devices and corresponded software packages, either a specific implementation, which accounts for the model structure and certain experimental tasks. *Figure 2.1b*) illustrates the constructed model with different types of implemented connections between units.

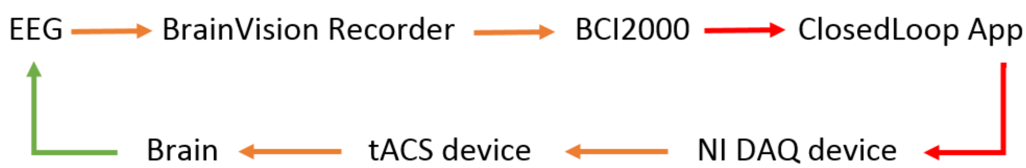


*Figure 2.1b Model scheme with highlighted connections: orange – configured by a standard parameters and functions, red – implemented with a custom code, green – defined by EEG amplifier and electrodes*

**Brain to EEG** connection is determined by a utilized amplifier and electrodes configuration. EEG data was recorded with BrainProducts amplifier BrainAmp MRplus (*Brain Products GmbH, Gilching, Germany*) with 31 Ag-AgCl electrodes mounted in an passive EEG EasyCap using a standard 10–20 system layout without Oz and Cz electrodes, with a reference and ground electrode positioned at FCz and AFz (*Figure 2.2*). Oz and Cz electrodes were excluded due to the same location of the stimulation electrodes.

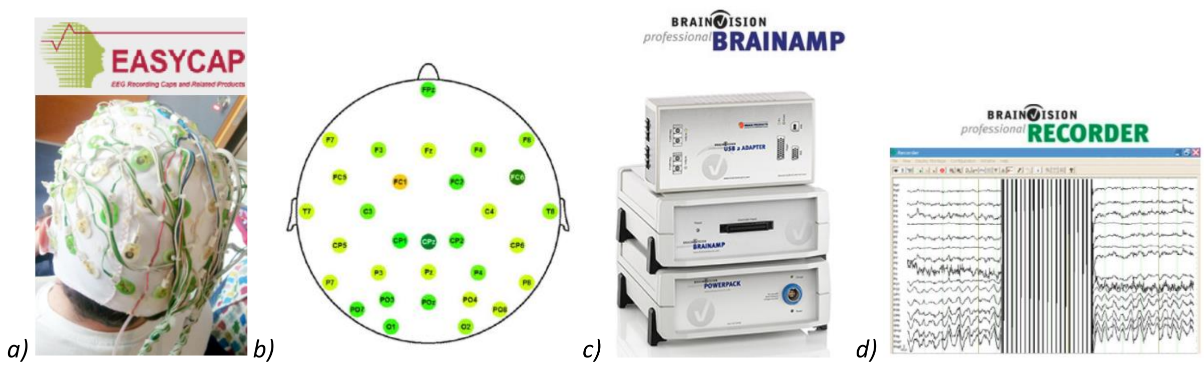


*Figure 2.2 a) passive EEG EasyCap b) electrodes montage scheme c) BrainAmp MRplus EEG amplifier d) BrainVision Recorder screenshot*

**EEG to BrainVision Recorder** connection is determined by a sampling rate frequency and filtering options, which are controlled by parameters in a workspace of BrainVision Recorder software, the sampling rate frequency was set to 500 Hz.

**BrainVision Recorder to BCI2000** connection is determined by RDA (remote data access) client of BrainVision Recorder for transmitting EEG data (to activate this mode – firstly *RemoteDataAccess* option should be enabled in Preferences window, then RDAClient should be run) and by corresponded signal source module of BCI2000 software for receiving the data, the transmission is arranged through TCP/IP protocol. Importantly, BCI2000 has a set of parameters for signal source module, which should strictly match parameters of a current workspace in BrainVision Recorder software. These parameters include: *number of channels* (31), *SampleBlockSize* – the number of samples transmitted at a time (10), *sampling rate* (500 Hz), *RDAClientADC* – the name of the host to connect to (localhost), *TransmitChList* – list of transmitted channels (28th – POz channel for extraction of the phase values).

**BCI2000 to ClosedLoop App** connection (*Figure 2.3*) is determined by UDP signal streaming option of BCI2000 and modified AppConnector interface example provided with BCI2000 [https://www.bci2000.org/mediawiki/index.php/Technical_Reference:App_Connector], which was adapted and incorporated as a class for data acquisition in ClosedLoop App. Importantly, an object of this class and its function for receiving the data was implemented in a separate thread to guarantee stable and fast signal processing without slowing down the functioning of the main thread with the graphical interface and all control elements.

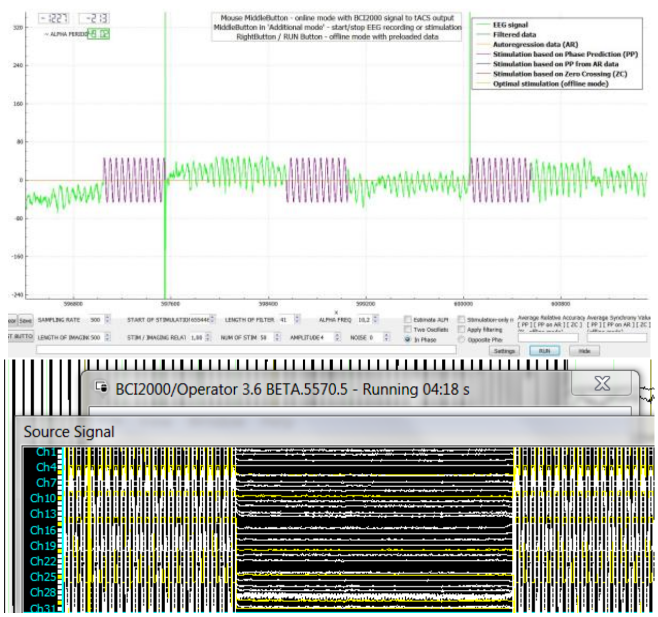


*Figure 2.3 (top) ClosedLoop App screenshot (down) BCI2000 source signal window*

**ClosedLoop App to NI DAQ device** connection is determined by a USB-cable from a laptop (*Lenovo ThinkPad P70, Intel Core i7, 16 Gb RAM*) to DAQ device, National Instruments drivers and custom-written C++ code using following functions from NI-DAQmx official ANSI C library:

```
DAQmxCreateTask(), DAQmxCreateAOVoltageChan(), DAQmxCfgSampClkTiming(),
DAQmxStartTask(), DAQmxStopTask(), DAQmxWriteAnalogF64()
```

**NI DAQ device to tACS device** connection (*Figure 2.4*) is determined by a BNC-cable and `AnalogOutput` mode of NI DAQ device and the REMOTE mode of tACS device. The REMOTE mode enables to operate the tACS device externally controlled by a voltage supply source (NI DAQ device), the generated current follows proportionally to the applied voltage. The official manual for DC-STIMULATOR PLUS explains the conversion principle from volts to mA: "using an external voltage supply source voltages between $U_{in} = -2 \,..\, +2\, [V]$ will be applied to the DC-STIMULATOR PLUS, out of this the DC-STIMULATOR PLUS will generate a proportional current $I_{out} = -4..+4\, [mA]$ according to the transfer function: $I_{out}\, [mA] = 2 * U_{in}\, [V]$." In REMOTE mode, direct, alternating and arbitrary voltage signal can be processed. The proportionality of the current amplitude relative to the externally supplied voltage can be guaranteed up to a frequency of 300 Hz, a linear transfer characteristic is guaranteed for voltages smaller than - 2 $[V]$ or greater than 2 $[V]$. In the current project during experimental studies a targeted current intensity for tACS was 1 $[mA]$ peak-to-peak, therefore, following the conversion principle, the input oscillating signal should have values in [- 0.5 .. + 0.5] $[V]$.

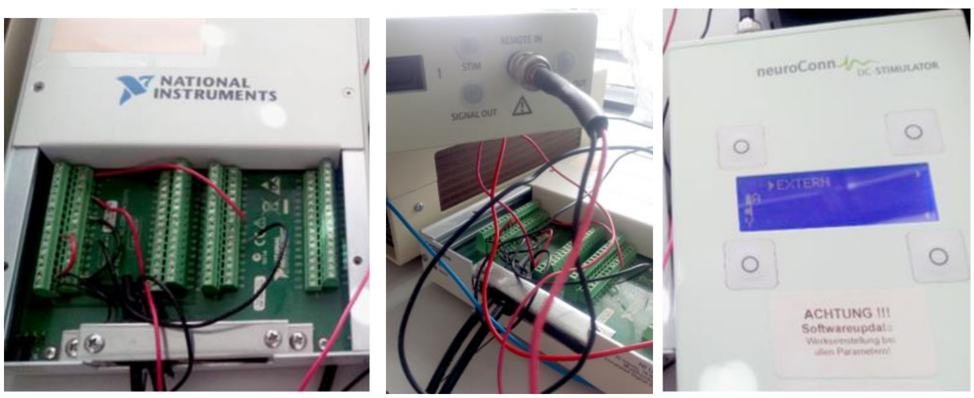


*Figure 2.4 (left) National Instrument USB 6229 device (DAQ device), (center) REMOTE IN input of DC-STIMULATOR PLUS with attached BNC cable from DAQ device, (right) DC-STIMULATOR PLUS*

**tACS device to Brain** connection is determined by stimulation electrodes montage (2 rubber electrodes 40x40 mm, located over Oz and Cz channels in 10-20 system), duration and intensity of the stimulation as well as frequency and phase of the stimulation. The targeted current intensity was 1 $[mA]$, other parameters were chosen according to a particular experimental protocol (the duration), individually estimated from resting state EEG recording before the stimulation (the frequency) and adaptively predicted from the data obtained on the intervals between the stimulation (the phase).

## *Functional modes*

The core control component of the whole system is ClosedLoop application. In general, the application has 3 modes of functioning: “Online”, “Offline”, “Record-only” / “Stimulation-only”. In “Online” mode (*Figure 2.5*) the system functions in a state required for adaptive stimulation with cycles of short interval (e.g. 1 sec) and incorporates: import of EEG signals (*imaging or pre-stimulation interval*), estimation of the optimal parameters and calculation of corresponded stimulation signal, computation of the required optimization time, compensation of optimization and transduction delays and transmission of the stimulation signal (*stimulation interval*) through NI DAQ card to stimulator device input (BNC port). A typical functional cycle during experimental session also contains *post-stimulation* interval for analysis of stimulation effects and a short inter-trial interval.

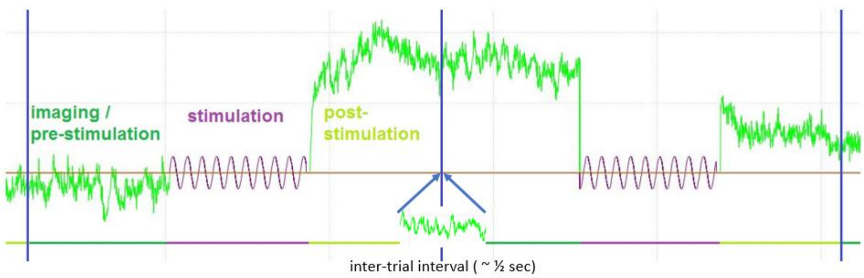


*Figure 2.5 Fragment of ClosedLoop Application screen in “Online” mode, length of all intervals – 1 sec, sampling rate = 500 Hz, green – EEG data from a participant (closed eyes, POz channel), violet – predicted in-phase stimulations (schematic representation without actual scale correction), blue lines – borders of trials*

“Offline” mode (*Figure 2.6*) is used for analysis of phase prediction methods and testing of the optimization procedures on predefined EEG datasets from human subjects and artificially generated EEG data. This mode does not generate alternating currents and has no post-stimulation interval, instead, there is an EEG data available within stimulation interval, which allows to compare phase prediction with actual phase of the alpha wave on this interval.

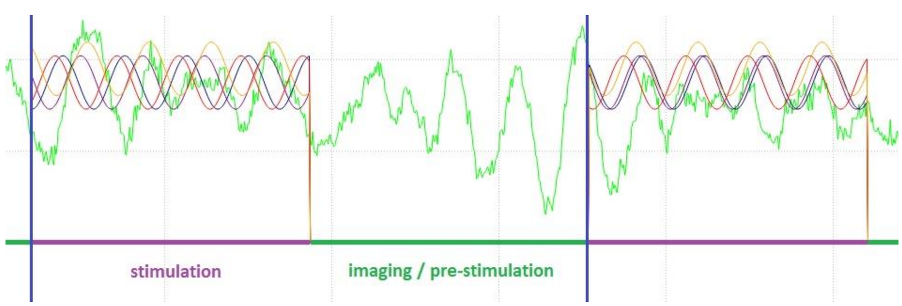


*Figure 2.6 Fragment of ClosedLoop Application screen in “Offline” mode, length of all intervals – ½ sec, sampling rate = 500 Hz, green – EEG data from a participant (closed eyes, Oz channel), red, blue, violet – predicted in-phase stimulations by different methods, orange – “optimal” stimulation*

In “Record-only” mode EEG signal is recorded and saved for further analysis. In “Stimulation-only” mode a particular number of stimulation stimuli with a predefined fixed parameters are transferred to the tACS device, this mode can be used for a conventional non-adaptive tACS.