User Guide for HelioScan 3

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List of abbrevations

AOD	acousto-optic deflector
GUI	graphical user interface
IMAQ	Image Acquisition driver package from National Instruments
NI	National Instruments
SSD	solid state disk
TIFF	tagged image file format
TLC	top-level component
VI	virtual instrument

1 HelioScan License

1.1 Terms

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2 Introduction

This user guide will help you to install, configure and use HelioScan, a software package for controlling microscopes. If you want to start extending HelioScan by developing your own components, please read the Developer Guide.

In this user guide, we assume that you already have acquired a basic understanding of HelioScan by reading the publication about HelioScan in the *Journal of Neuroscience Methods* [2]. If you miss any information in this manual, please also consult [1]. In case of further questions, don't hesitate to contact the responsible authors of this manual:

- *Dominik Langer:* General architecture of HelioScan, intrinsic optical imaging, laser scanning with galvanometric scan mirrors
- *Marcel van 't Hoff:* Laser scanning based on acousto-optic deflectors (AODs)
- *Andreas Keller:* Laser scanning based on a resonant scanner
- *Marco Tedaldi:* Data acquisition with a National Instruments DAQ card
- *Hansjörg Kasper:* Signal quality and noise issues

3 Requirements

3.1 Hardware requirements

3.1.1 Set-up PC

LabVIEW allows to easily program code that can be executed in parallel and HelioScan makes extensive use of this feature. Therefore, we recommend to use an up-to-date PC with at least four processor cores. For fast start-up of HelioScan and saving of acquired data, the operating system as well as HelioScan is optimally installed on a solid state disk (SSD). To allow the main graphical user interface (GUI) of HelioScan to be properly displayed, a monitor with a resolution of at least 1600x1200 pixels is required.

Example configuration

At the time of writing, the following hardware combination provided to work well:

- ASUS P6T DELUXE motherboard
- Intel Core i7-950 3.06 GHz processor (quadcore)
- 12 GB DDR3 1333 MHz RAM
- NVIDIA GTX460 1GB graphics controller
- 1TB S-ATAII harddisk (7200 rpm)
- Kingston SV100S2/128G solid-state disk with 128GB
- DVD-+R/RW dual-layer drive
- PCI-Express serial port (RS-232) card

3.1.2 Periphery hardware

The type of periphery hardware needed depends on several factors. These include your particular type of microscope (e.g. which stage device it is based on), its image acquisition modality (e.g. laser-scanning with a particular combination of scanner types), the imaging modes you want to use (e.g. 3D spiral scanning), the experiments you want to perform (you might need specific hardware for stimulators) and the available budget.

3.2 Software requirements

Prior to installing HelioScan on your set-up PC, make sure that you have installed the following software:

- Microsoft *Windows 7* operating system (64bit version is recommended, but not required)
- National Instruments *LabVIEW 2010 SP1*
- National Instruments *Vision Development Module* for LabVIEW 2010
- National Instruments *FPGA Module* for LabVIEW 2010 (only required if you plan to use FPGA-based signal acquisition or generation
- National Instruments *Device Drivers* (newest version)

- JKI *VI Package Manager* (community edition)
- JKI *easyXML* (license required)

4 Installing HelioScan

4.1 Registering

You need to register before you will be able to download the HelioScan software. Please proceed as follows:

1. Create a GitHub account[1]. Please choose a human-recognizable user name (i.e., for example rather not *bfg2009*, but preferrably something like *dominik.langer*). This will help us to better keep track of the HelioScan user base.

2. Write an email to one of the first two authors of this manual and ask to be added to the HelioScan project at GitHub. Don't forget to mention your user name.

4.2 Downloading

The newest version of HelioScan can be downloaded as a ZIP file from the HelioScan website[2]. After unzipping, copy the HelioScan directory to an appropriate location on your disk drive. We recommend to copy it directly to the root directory of the drive to have it quickly accessible for all users.

We also recommend that you keep HelioScan in a folder that reflects the version number (e.g. c:\helioscan-3.2.10). In particular if you have multiple versions of HelioScan installed, this will allow you easily distinguish between them.

[1] `https://github.com`
[2] `http://www.helioscan.org`

5 Configuring HelioScan

A HelioScan application assembles at run time from individual components. The HelioScan main virtual instrument (VI) loads so-called top-level components (TLCs), which in turn may load subcomponents, which may again load their own subcomponents and so on. Which components are to be loaded as well as further parameters determining the run-time behaviour of components, is determined by configuration files. The user has to create these configuration files prior to use to customise HelioScan according to his microscope hardware as well as his experimental needs.

5.1 Preparing steps

Navigate into the HelioScan directory and create two subdirectories named "configuration" and "settings". Inside the configuration subdirectory, create a directory named "default". This directory will hold all configuration files for a default configuration of HelioScan. If you require different configurations for different users, create a subfolder for each user inside the configuration subdirectory.

5.2 Configuration wizard

Configuration wizards provide a structured path of configuring HelioScan for a specific purpose (e.g., frame scan mode with galvanometric mirrors). They assist the user in creating configuration files by assuming a specific combination of software components and guiding through a series of steps. The use of a configuration wizard provides a good starting point, especially for the less experienced user.

5.3 Configuration manager

5.4 Configuration files

6 General aspects of using HelioScan

6.1 Starting up HelioScan

The HelioScan main VI (HelioScan_3.vi) cannot be started directly from within the Windows Explorer, but rather needs to be started from within HelioScan's LabVIEW project browser.

1. If you have multiple LabVIEW versions installed on your set-up computer, start LabVIEW 2010 SP1 and close all other running LabVIEW versions if possible.

2. Double-click on the HelioScan project file (helioscan.lvproj) in the HelioScan base directory. The LabVIEW project browser window will open, showing you a tree view of project items. Note that the project browser will open in the most recently started LabVIEW version. For HelioScan to run properly, this needs to be LabVIEW 2010 SP1. If you require running another LabVIEW version simultaneously for some reason, open the HelioScan project file directly from within LabVIEW 2010 SP1 in order to prevent that it is opening in the wrong LabVIEW version.

3. Within the project browser, open the HelioScan main VI by double-clicking on the item Helioscan_3.vi.

4. Start the HelioScan main VI via the VI start button. If it is the first time you are starting up a newly installed HelioScan version, the start-up process might last several minutes because LabVIEW compiles all HelioScan files being loaded. Once compiled, starting up HelioScan typically requires between half a minute and a full minute to load its initial combination of components. During the start-up process, a progress bar indicates the progressive loading of components. Loading is finished and HelioScan ready for use, when the progress bar has completed and switched its colour from orange to green.

6.2 Shutting down HelioScan

There are several ways to shut down HelioScan. Here, they are listed with increasing harshness. We recommend to start with the softest method and work your way down the list in case you are not successful. Due to the complex nature of a running HelioScan application and its dependency on other software and hardware resources this might sometimes be necessary.

- The recommended way to terminate the HelioScan main VI is via the HelioScan exit button. This will properly terminate all components and release all resources including allocated memory and hardware resources, as indicated by the progress bar. The main VI will stay open so you can re-start it if needed. In case the main VI keeps hanging during shut-down, proceed with the next harsher method to shut down.

- Terminate the HelioScan main VI by clicking its VI stop button. This will not execute the shut-down procedures of the individual components, but stop the main VI immediately.

It will still stay open so you can re-start if needed. If the main VI does not react when you press the VI stop button, proceed with the next harsher method.

- Terminate LabVIEW including HelioScan by killing the LabVIEW process in the Windows Task Manager. This is to fastest method to shut-down HelioScan. If you want to restart, you need to proceed as outlined in Section 6.1.

6.3 GUI of the HelioScan main VI

You need to be familiar with the following user interface items of the HelioScan main VI and their respective terms:

- *TLC tabs:* Each TLC owns a dedicated tab into which the respective TLC's GUIs is loaded at run-time. An exception is the GUI of the Display TLC, which is not loaded into a TLC tab, but owns its own area in the upper half of the main VI's GUI.
- *Progress bar:* A multi-colour progress bar indicates the progressive loading and unloading of TLCs and the readiness of the main VI. If all TLCs are loaded and initialised, the progress bar is at 100 % and indicates via its green colour that HelioScan is ready for imaging. If any of the TLCs is missing, the colour is orange, thus signalling that HelioScan is not ready for imaging yet.
- *User selector*: This drop-down menu allows choosing between different configuration sets. Basically, each user can have its own set of configuration files stored in subdirectories of the HelioScan configuration folder (see Section 5.1). The names of these subfolders are listed in the drop-down menu and are available for selection. By default, the configuration set contained in the folder "default" is selected.
- *Objective selector*: This drop-down menu allows selecting an objective. Use the main configuration dialog of HelioScan to configure which objectives are available for selection. There, you can also configure the default value of the objective selector. For two reasons, we recommend that you always select the objective that you have effectively in use for your measurement. First, the objective properties (most importantly, it's magnification factor) are usually stored in the meta-information section of image files generated by HelioScan (at least of you use a DataCollection TLC. Second, the magnification factor of the selected objective is needed for the calculation of the scale bar that can be displayed as an overlay on the image display.
- *ExperimentController selector:* This drop-down menu lists all the ExperimentController configurations added in the HelioScan main configuration dialog. It allows selecting the ExperimentController instance to be used when imaging in *experiment mode* (see Section 6.4).
- *ImagingMode selector:* This drop-down menu lists all ImagingMode configurations added in the HelioScan main configuration dialog. It allows selecting the ImagingMode instance to be used for imaging. When imaging in *experiment mode* (see Section 6.4), it is important to be aware that certain ExperimentControllers can be configured to automatically choose different ImagingModes or ImagingMode configurations in automated imaging sequences. Other ExperimentControllers always use the ImagingMode that you

have previously selected using the ImagingMode selector. When imaging in *single-sweep mode*, HelioScan always uses the ImagingMode configuration that you specified via the ImagingMode selector.

- *Start/stop buttons:* HelioScan offers three different buttons to start and stop image acquisition. Each button is responsible for a different run mode (see Section 6.4). From top to bottom, these are the start/stop button for *focus mode*, *single sweep mode* and *experiment mode.*

- *Save button:* Use this button to actively trigger saving of the data you have acquired. In *single-sweep mode*, data acquired during the last sweep will be saved. In *experiment mode*, either data of the last sweep or of the whole sweep series will be saved (depending on the DataCollection TLC you are using). Note that certain DataCollection TLCs give you the option to enable auto-saving, such that each sweep is automatically saved and you do not need to invoke the save button.

- *Version indicator:* This indicator displays you are current version of HelioScan (note that the indicator is dynamically updated during the start-up process, so do not rely on its content before the progress bar is in status *ready.* The indicator also shows (via an orange exclamation mark) when a more recent HelioScan version is available for download.

- *Notebook:* The notebook logs image acquisition details during a measurement session. Notebook log files are automatically and incrementally saved whenever the notebook content changes. This means that for a single imaging session, you typically end up with a large number of notebook files. Since log files names contain a time stamp, you can sort them according to name in Windows Explorer and easily find the most recent version. When you accidentally made an unwanted change to the notebook, you can go back to earlier versions to recover the original information. When saving data to disk, HelioScan typically adds the name of the save together with some more acquisition details. You are free to manually enter further information anywhere in the already existing notebook content.

- *Exit button:* This button should be used to shut down HelioScan at the end of a imaging session (see Section 6.2).

6.4 Run modes

HelioScan performs image acquisition in one of three run-modes. You as the user decides which run mode is to be used by pressing the corresponding start button (see Section 6.3).

- *Focus mode:* In this run mode, image acquisition continues until stopped again by the user via the corresponding start/stop button. Use this run mode to find you region of interest in the specimen and to adjust imaging conditions (such as illumination power).

- *Single-sweep mode:* In this run mode, image acquisition continues for the number of frames or duration specified in the currently active ImagingMode. Acquired images are temporarily stored until you press the safe button or start a new sweep.

- *Experiment mode:* In this run mode, control over image acquisition is handed over to the currently active ExperimentController TLC. The ExperimentController is typically used to perform a series of sweeps in a row. Depending on the ExperimentController used, sweep acquisition may for expample be started in regular time intervals or upon hardware triggers.

7 Trouble shooting

7.1 Reporting issues

8 Video camera imaging

8.1 Hardware requirements

8.2 Configuration

8.2.1 Recommended configuration parameters

9 Intrinsic optical imaging

9.1 Hardware requirements

9.1.1 Image acquisition

To perform intrinsic optical image with HelioScan, you need a camera and a way to interface the camera to your set-up PC that is supported by the National Instruments IMAQ driver package[1].

Since the intrinsic optical signal is very small (in the range of half a promille reflectance change), it is essential to have a camera that can discriminate a high number of brightness levels per pixel. A black-and-white camera with 12 bits per pixel is recommended; it can in principle resolve 4096 grey levels, corresponding to an intensity change of 0.2 ‰ from one level to the next.

Package A

This package has been extensively used at the laboratory of Fritjof Helmchen and has proven to work well. The camera is not offered anymore by Toshiba Teli, however.

- Teli CS3960DCL camera (12 bit per pixel; from Toshiba Teli Corporation)
- NI PCI-1426 camera link card (from National Instruments)

Package B

- Basler avA1000-120km (12 bit per pixel; from Basler AG)[2]
- NI PCI-1426 camera link card (from National Instruments)

9.1.2 Illumination

In order to visualise the blood vessel pattern, which serves as a reference map to localise the intrinsic optical signal, green light is optimally used. For the acquisition of the intrinsic optical signal, red light is better suited. It is thus useful to have the possibility to switch between green and red light, for example by using a ring with two types of LEDs, such as:

- *Green light:* L5-G61N-GT (peak wavelength 525 nm; from Sloan LED)
- *Red light:* L-7113SRD (peak wavelength 660 nm; from Kingbrith Electronic)

9.1.3 Optics

Intrinsic optical imaging can be performed directly using an existing in vivo two-photon microscope equipped with a suitable camera. Alternatively, one can built a stand-alone setup for intrinsic optical imaging [1].

[1]The *Industrial Camera Advisor* from National Instruments can help you identifying a suitable device: `sine.ni.com/apps/utf8/nipc.specs?action=search&asid=1102`

[2]For details, see `http://www.graftek.com/pages/avA1000-120km.htm`

Integration into a two-photon microscope

To image the back aperture of the microscope objective, an appropriate objective on the camera side is required. A camera objective with zoom functionality is well-suited, such as for example:

- Monacor 5-50mm 1:1.4 1/3” CS

9.1.4 Stand-alone intrinsic opical imaging set-up

9.2 Configuration

Please configure first the video camera mode, as it is required to acquire the reference image of the brain surface vasculature Section 9.3. Afterwards, continue with configuring the actual intrinsic optical imaging mode as described here.

We recommend to use the configuration wizard (see Section 5.2) with the wizard file *intrinsic_imaging*.

9.2.1 Recommended configuration parameters

The following configuration parameters can be used.

DAQ_MG090622Camera

- *Device name:* look it up in the Measurement & Automation Controller
- *Camera type:* gray scale
- *Connection type:* depending on how you connected the camera to your set-up PC (select "CameraLink" in case of the two packages listed in Section 9.1.1).

DataCollectionMG091001IntrinsicImaging

- *Multiplication factor:* 100000

ImageProcessorDL090216RangeOffset

- *Maximum exptected value:* 0.05

DisplayDL090216

- *Number of channels:* 1
- *Range-and-offset image processor:* intrinsic

ImagingModeMG091111IntrinsicImaging

- *Instrument:* leave empty
- *DAQ:* "DAQ_MG090622Camera.lvclass with configuration "intrinsic"
- *ImageAssembler:* "ImageAssemblerMG090623Binning.lvclass" (with empty configuration)
- *DataCollection:* "DataCollectionMG091001IntrinsicImaging.lvclass" with configuration "standard"

- *Display:* "DisplayDL090216.lvclass" with configuration "intrinsic"
- *Analyser:* "AnalyserDL090623TwoD.lvclass" with configuration "intrinsic"
- *Stimulator:* leave empty; needs to be configured separately.

ExperimentController

- *Class file and configuration of Sweep component:* leave empty

Main configuration

- *ExperimentControllers:* add "ExperimentController090130Interval.lvclass" with configuration "intrinsic"
- *ImagingModes:* add "ImagingMode091111IntrinsicImaging.lvclass" with configuration "standard"
- *Objectives:* Create a configuration for each objective you want to use via the button "Manage objective...". Afterwards, add each objective via the button "Add/remove objectives...".
- *Stage:* leave empty, unless you have already specified a Stage while configuring another mode

After finishing the configuration wizard, you need to separately configure your Stimulator TLC of choice. We recommend to use the configuration manager for this purpose (see Section 5.3). Also using the Configuration Manager, open again the configuration you created for `ImagingModeMG091111IntrinsicImaging` and specify the specific Stimulator TLC you just configured, including its newly created configuration.

9.3 Experimental protocol

9.3.1 Animal preparation

Details of animal preparation are not in the scope of this software manual and need to be read elsewhere (TODO: refs). Here, we only want to stress the three most important points regarding preparations for intrinsic optical imaging:

- *Transparency:* You need optical access to the brain. Depending on the animal species and age as well as other experimental constraints, you might achieve transparency by a craniotomy above the area of interest or by local thinning of the skull. In the latter case, it is essential to keep the remaining thin layer of skull wet, for example by filling a local chamber above the skull with Ringer solution and thighly sealing with a cover slip and grease. In young mice, merely removing the sking might be sufficient; the Ringer solution might induce enough transparency.

- *Mechanical stability:* It is crucial that you reduce breathing- and heart-beat-induced movements of the brain surface to the minimum. In this respect, skull thinning may have an advantage over a complete craniotomy. In any case, we recommend locally covering the skull around the area of interest with a layer of agarose, slightly pressed down by a microscopy cover slip. In order to keep the agarose.

- *Depth of anaesthesia:* Aneasthesia should be as slight as possible without the animal suffering pain or breathing unsteadily.

9.3.2 Acquiring a blood vessel map

At the very beginning of the imaging session, a reference image of the brain surface vasculature needs to be acquired. For this purpose, illumination giving the highest possible contrast between blood vessels and neural tissue is required (green light is well suited). The recommended procedure is as follows.

1. Mount a low magnification objective (e.g., 4x or 10x) and switch to green light illumination.
2. Select the video camera ImagingMode using the ImagingMode selector.
3. Adjust the illumination power in *focus mode* to achieve good contrast and avoid saturation.
4. Acquire a high-resolution image of the blood vasculature in *single-sweep mode* (e.g. 1000x1000 pixels; obviously, the resolution you choose needs to be supported by your camera). If necessary, this image may serve you to zoom into the actual reference map acquired in the next step.
5. Acquire an image at the same resolution (see Section **??**) later used for acquiring the intrinsic optical signal. This is the actual reference map on which you will later overlay the intrinsic optical signal.

9.3.3 Static mapping of the intrinsic optical signal

Now that you are equipped with a reference image with a blood vasculature map, you are ready to record the intrinsic optical signal. Intrinsic optical imaging requires a lot of signal averaging. You will perform measurements in *experiment mode*, with the ExperimentController performing multiple acquisition sweeps for you. Since you did not specify a particular Sweep class (see Section 9.2.1), the currently selected ImagingMode will be used. The DataColleciton will do the signal averaging for you.

The recommended procedure is as follows:

1. Switch to illumination suitable for intrinsic optical imaging (we recommend red light illumination).
2. Select the ImagingMode for intrinsic optical imaging in the ImagingMode selector.
3. Specify the ImagingMode parameters. The following values have proven to work well for static mapping experiments: a phase duration of 5.0 s, an offset time of 0.5 s, an image resolution of 300x300 pixels, and a frame rate of 5.0 Hz. If you are not interested in a control measurement without stimulation, you can enable the check box *skip control*. HelioScan will then skip acquisition phase A and directly start with acquisition phase B, thus saving 5 s in case of the parameters mentioned above. We do not recommend to do so, however, since the control measurement allows you to distinguish the actual intrinsic signal more easily from other structures visible on your resulting images.
4. Specifiy the ExperimentController parameters. Start with 15 sweeps repeated with a sweep period of 35 s[3] The latter should give the intrinsic optical signal enough time to

[3]The sweep period must be no shorter than the total sum of a the three (or two, if you ommit the control measurement) phases plus the acquisition offset.

return to base line after a sweep before the next one is started. If you receive good results, you can tweak these parameters in order to save time, for example by reducing the number of sweep and/or the sweep period.

5. Specify the Stimulator parameters. These depend on the actual Stimulator class chosen during the configuration process (see Section 9.2). In any case, it is crucial that stimulation starts only after acquisition phase B has finished. In case of a acqusition phase duration of 5.0 s, this would correspond to a stimulation offset of 10.0 s. Also, you want to stimulate during the entire acquisition phase C. With a phase duration of 5.0 s and an acquisition offset of 0.5 s, a stimulation duration of 5.5 s is required for this purpose.

6. If you want to save the acquired data after each sweep, go select the DataCollection TLC tab and select the option "autosave sweeps on the fly". This will give you the option to analyse already acquired data offline in case of a hardware or software crash to could prevent you from saving after your experiment has been finished.

7. Start acquisition in *experiment mode* and wait until all sweeps have been acquired.

8. Select the DataCollection tab to inspect the acquired data. For static mapping of the intrinsic optical signal, select the check boxes "average over time" and "average over sweeps". Switch between the sweep parts "actual measurment" and "control" using the corresponding drop-down menu.

9. Specify the saving parameters and push the save button. You can inspect the generated tagged image file format (TIFF) files using ImageJ.

10. Repeat the experiment for all stimulation conditions you want to map (e.g., for all whiskers you want to map on the rodent barrel field).

11. Using the overlay tool (accessible via the button "Overlay..." on the DataCollection GUI, you can mark the mapped brain regions on the blood vessel map acquired in Section 9.3.2.

9.3.4 Recording the time course of the intrinsic optical signal

9.4 Data analysis

All data saved during an experiment can be found in the HelioScan data folder, in a subdirectory with the time of saving as its name. Pixel values in the generated TIFF files are multiplied with factor and added with an offset in order to fit into the range of 0 to 65385 (16 bit integer data format). Factor and offset and stored in the notebook and part of the file name as well.

The overlay tool mentioned in Section 9.3.3 can also be started independent from HelioScan. It can found in the folder *tools\IntrinsicAnalyzerMG100121*.

Bibliography

[1] D. Langer. *A flexible software framework and post hoc cell type discrimination for in vivo two-photon calcium imaging of neuronal population activity.* Phd thesis, 2011.

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