Acquisition

ContImage - CONTinuousIMAGE

ContImage controls the CCD camera, acquires frames, displays frames, saves preprocessed frames, previews green and exposure images.

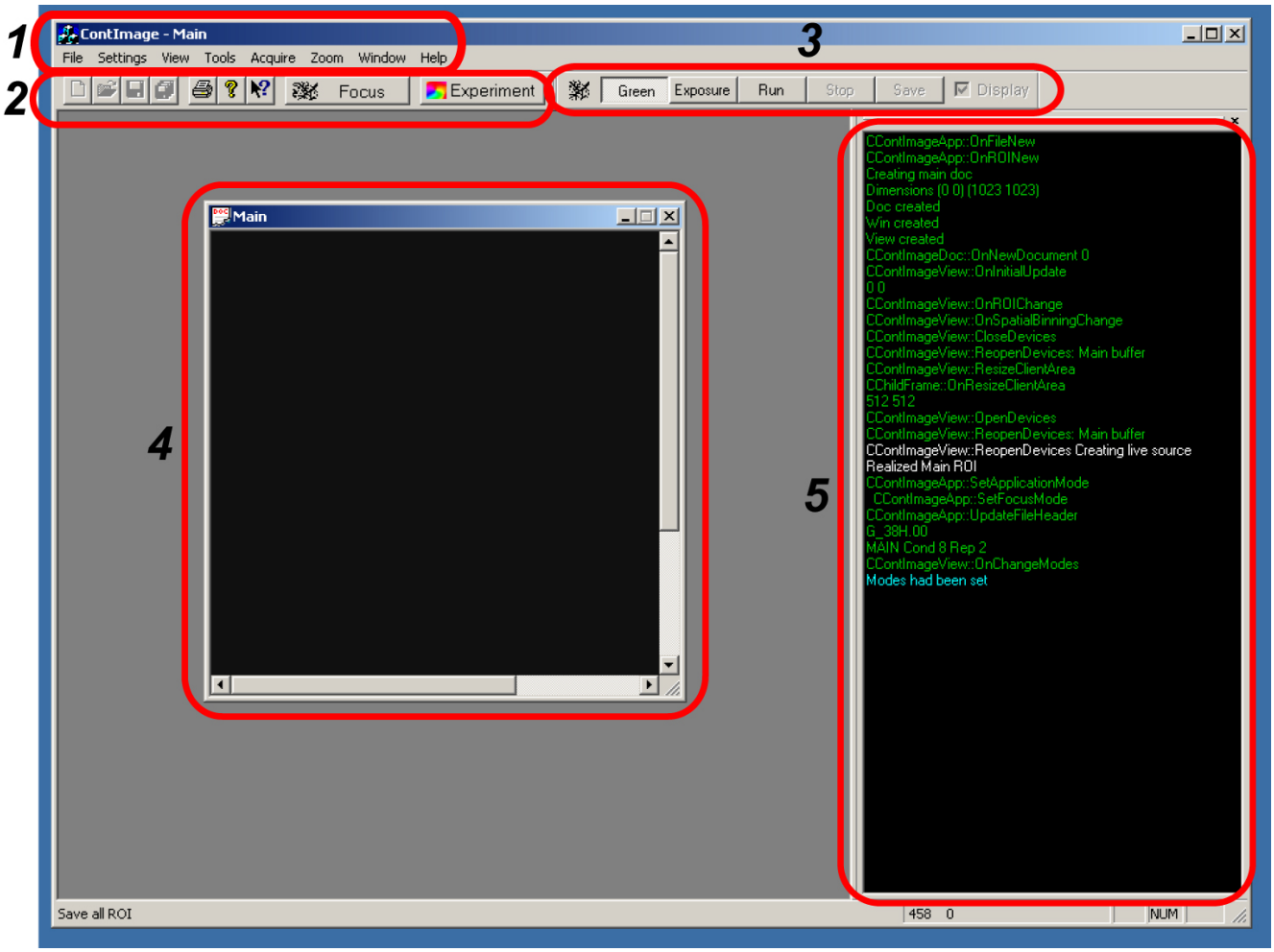


Figure 1. *Components of ContImage.*

Components of ContImage

1 Menu bar. Function: selection of controls and functions.

2 Main tool bar. Function: opening and saving images, switching between Focus and Experiment modes of operation)

3 Control tool bar. Function: starting, stopping, aborting, frame acquisition, saving images, switching between Green and Exposure sub-mode in the Focus mode

4 Image window. Function: displays acquired frames.

5 Log window. Function: error (red) and warning (cyan) logging. Other types of logged info are colored white for normal events, green and magenta for debugging messages. The latter ones can be ignored.

Other components include setting dialog boxes (evoked from the "Settings" button of the Menu bar) and "Histogram Tool" and "Trace Tool" (evoked from the "Tools" button of the Menu bar).

ContImage: Modes of Operation

There are two functional modes of operation: **Focus** and **Experiment**. The main menu of the program is used to switch between the modes. Each mode has sub-modes of operation and its own control toll bar.

ContImage Focus Mode

Focus mode is used to find and select a region of interest (ROI), to obtain image of cortical surface (vasculature) as a reference, and to evenly illuminate the surface. **Focus** mode is controlled via Focus tool bar (Figure 2). There are two sub-modes of operation: **Green** and **Exposure**. The Focus menu (see Figure 2) is used to switch between the sub-modes.

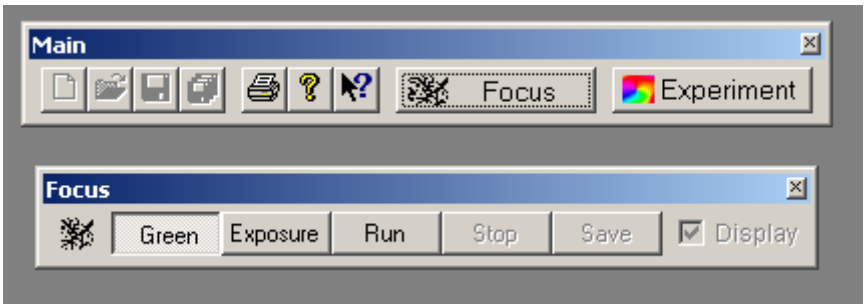


Figure 2. *Main tool bar (Focus mode selected) and Focus Control tool bar (Green sub-mode selected).*

Green sub-mode (green light is used for illumination) is used to focus camera on the ROI and to obtain the image of the surface. The displayed image is black and white (gray scale) (see Figure 3). **Exposure** sub-mode (red light used for illumination) is used to achieve even illumination of the ROI (see Figure 4). The displayed image is false-colored to emphasize small differences of the reflected light and to reveal areas where the camera pixels are saturated.

Both **Green** and **Exposure** sub-modes can be run in three view-modes: 1. **ROI**, 2. **Fast** (display only, main ROI only), and 3. **Experiment**. The desired view-mode can

be selected through Focus dialog box: Settings ⇨ Focus (see Figure 5). **ROI** view-mode is run without spatial and temporal binning. Images can be saved in this view-mode. Images transferred directly from the camera to the video memory in the **Fast** view-mode. This view-mode does not allow saving images and is used only for viewing purposes. The **Experiment** view-mode is employed to display images, as they will appear while running actual experiment, i.e., spatially and temporally binned. If no binning is used for the experiment the **ROI** and **Experiment** view-modes are identical.

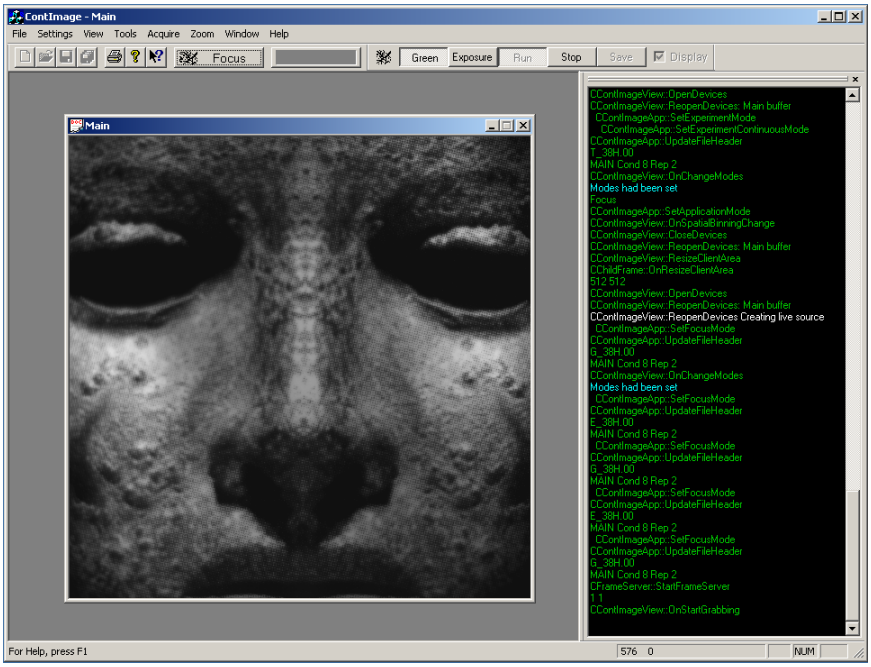


Figure 3. *ContImage running in Focus mode (Green button is pressed on the Focus Control tool bar). The view mode is Fast, that is why the Save button and Display box of the tool bar are grayed (dysfunctional).*

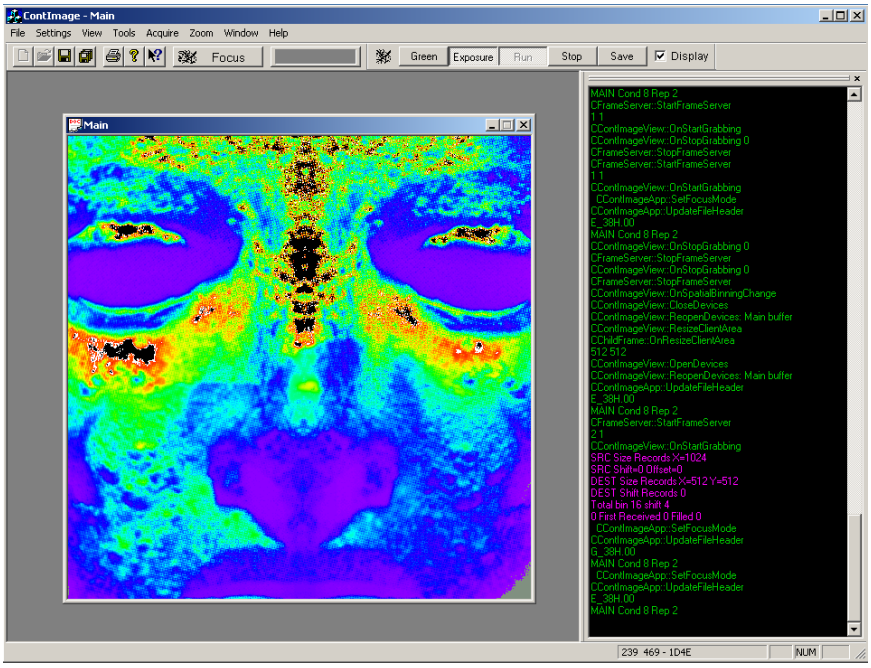
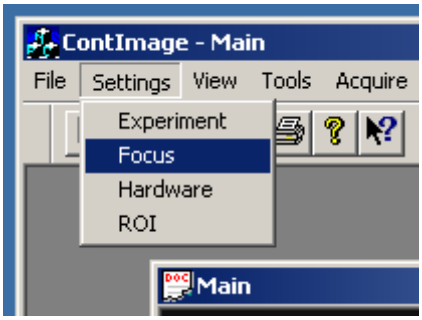


Figure 4. *ContImage running in Exposure mode. (Exposure button is pressed on the Focus Control tool bar). The view mode is ROI, that is why the Save button and Display box of the tool bar are functional. The blackened pixels indicate saturation and should be avoided.*



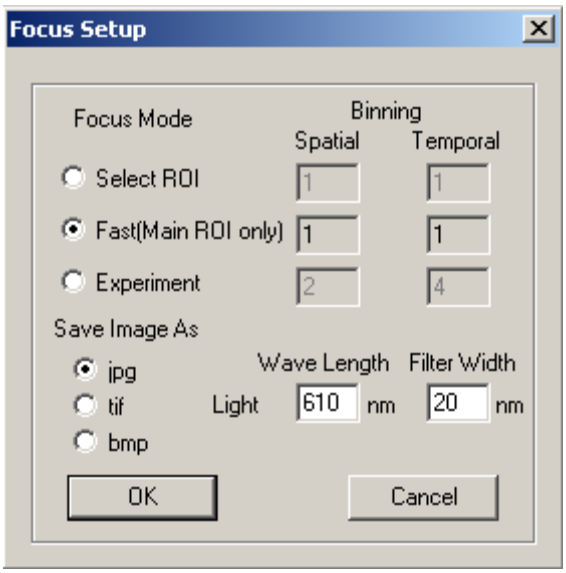


Figure 5*. Setting up the Focus mode. (Left panel) Selection of the "Focus Setup" dialog. (Right panel) "Focus Setup" dialog. Focus view mode is set to "Fast". Image save format is set to JPEG. Other optional info can be provided in the "Wave Length" and "Filter Width" fields.*

While running in **ROI** or **Experiment** view-modes a smaller rectangular ROIs can be selected with the mouse by clicking on and dragging the cursor (Figure 6A). When a new ROI is accepted (Figure 6B) a new window will pop up (Figure 7A). An index in the left-top corner identifies the smaller ROIs. The Main ROI (index 0) does not have the index displayed. Up to 31 smaller ROIs can be selected. It is recommended to close the Main ROI after selection of sub-ROIs is complete (Figure 7B) because the display of the Main ROI together with the sub-ROIs is CPU-time intensive. Leaving both the Main and sub-ROIs open in the Experiment mode is irrational since parts of the image saved with the sub-ROIs will have been saved with the Main ROI already.

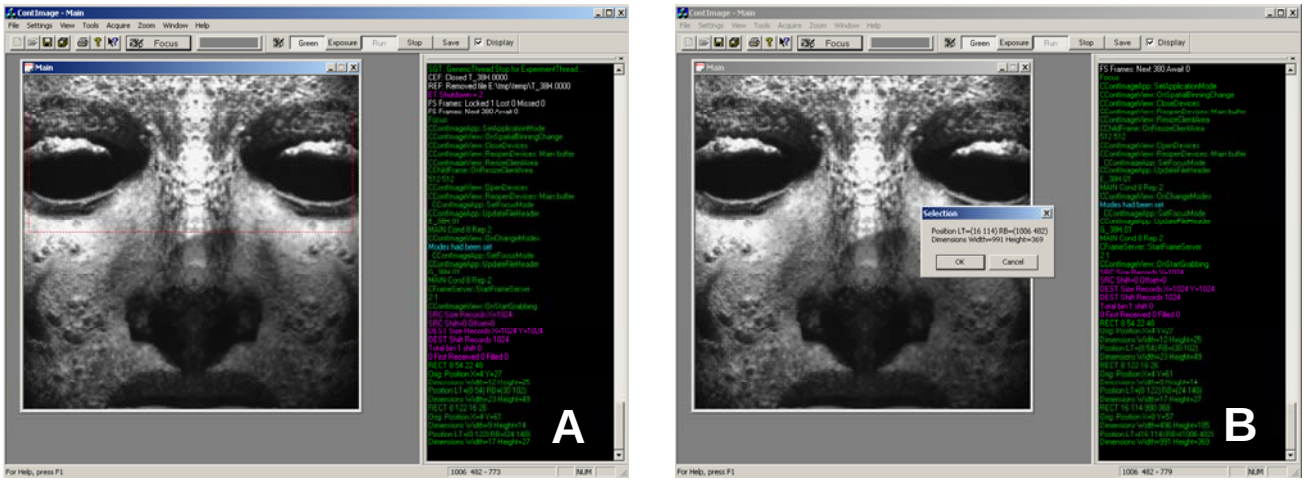


Figure 6*. Selection of smaller ROI. (A) Select rectangular region by clicking on and dragging the mouse cursor. (B) Accept/reject the selected region.*

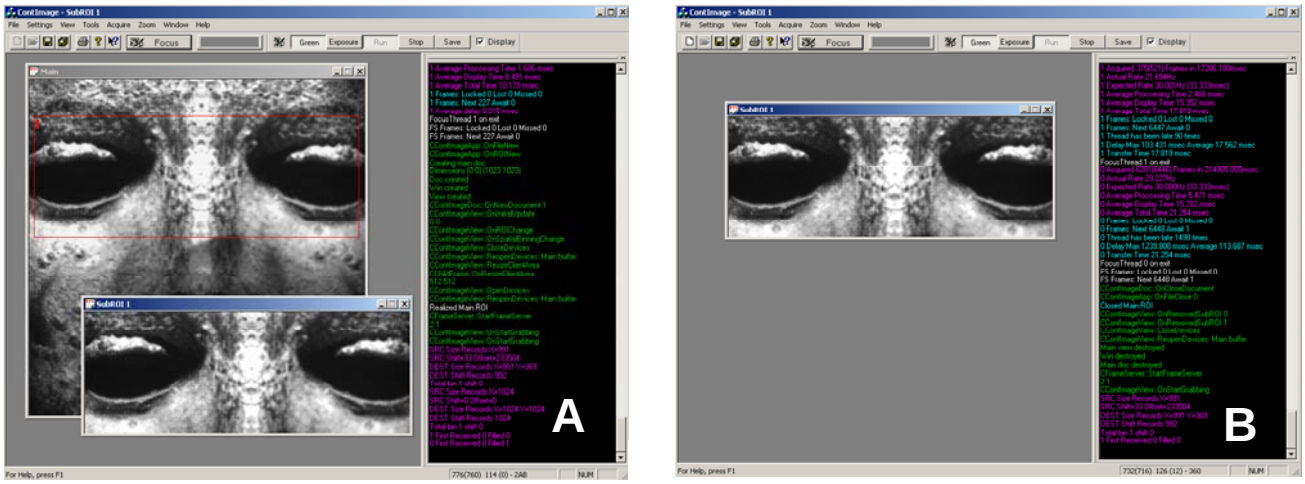


Figure 7*. Selection of smaller ROI. (A) Both Main and sub ROI windows are open. (B) The Main ROI window is closed (by clicking on the close button, top right "X" button of the respective window, or through File ⇨ Close).*

The Main ROI and/or selected sub-ROIs can be saved as raw frames by clicking on "Save" button of the Focus tool bar. These files are used by **iman** analysis program to produce PostScript files. The same images can be saved as JPEG files by clicking on "Save" or "Save All" Main tool bar buttons. Files cannot be saved in the **Fast** view-mode. It is recommended to save all images (Main and sub-ROIs) before closing the Main ROI.

ContImage Experiment Mode

Experiment mode is used to acquire, preprocess (bin), and save frames in real time, meaning that all frames of the continuous data stream are saved (only a few reference images are saved in the **Focus** mode). **Experiment** mode is controlled via Experiment tool bar (Figure 8).

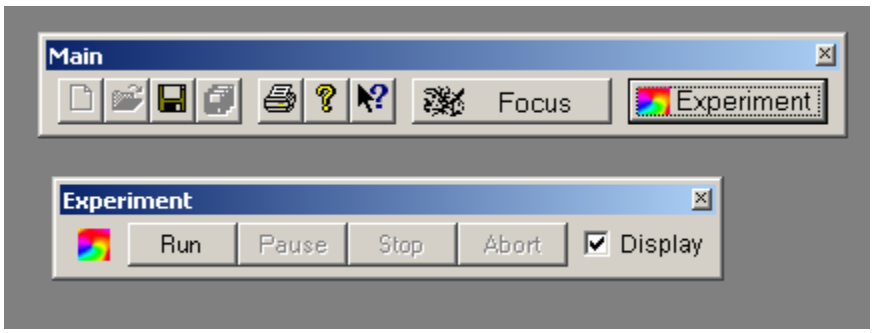


Figure 8. *Main tool bar (Experiment mode selected) and Experiment Control tool bar (Display box is checked).*

Description of the Experiment Setup Dialog box

Currently there are two types of experiments: **Continuous** and **Episodic**. Selection of the experiment type is made through the Experiment dialog box: Settings ⇨ Experiment (see Figure 9).

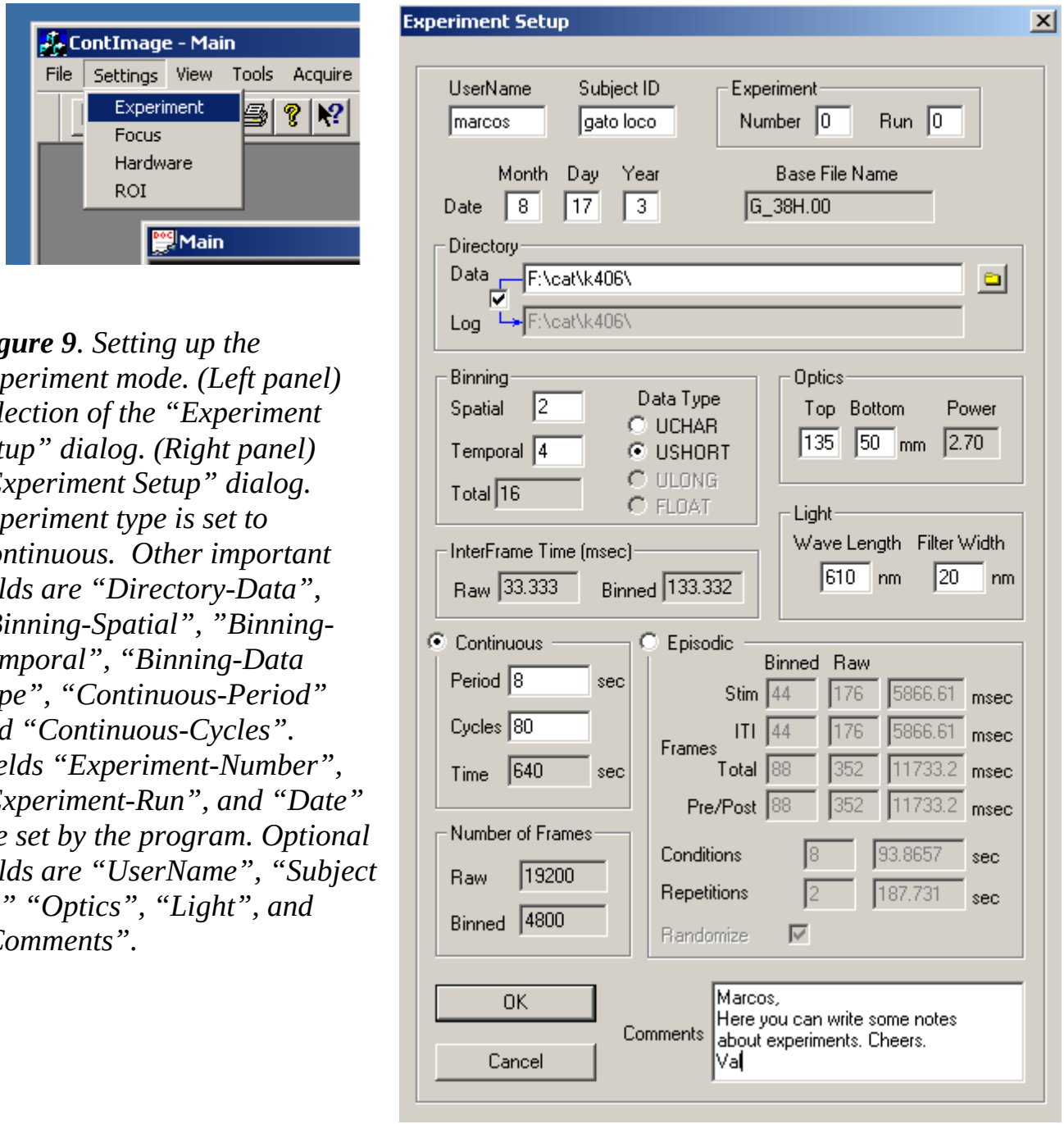


Figure 9*. Setting up the Experiment mode. (Left panel) Selection of the "Experiment Setup" dialog. (Right panel) "Experiment Setup" dialog. Experiment type is set to Continuous. Other important fields are "Directory-Data", "Binning-Spatial", "Binning-Temporal", "Binning-Data Type", "Continuous-Period" and "Continuous-Cycles". Fields "Experiment-Number", "Experiment-Run", and "Date" are set by the program. Optional fields are "UserName", "Subject ID" "Optics", "Light", and "Comments".*

ContImage was design for continuous-periodic imaging paradigm: **Continuous** experiment mode. Imaging can be done in the old-fashioned manner using **Episodic**

experiment mode. In the latter case **ContImage** is run in master mode sending control sequences via the DIO channel to the stimulus computer running PCV or other stimulus generator.

The white fields in the dialog box can be modified by the user or the program. The grayed fields (read-only) are computed from the values defined in the white ones. Important fields of the Experiment Setup dialog are:

Directory-Data. This field specifies where the experiment files will be saved. The data directory can be typed in or selected through File Select dialog by clicking on the folder button (right of the field).

Binning Spatial, Temporal, and Data Type. These fields define binning of the saved frames relative to the original frames. Binning allows strong reduction of the sizes of the saved files. The typical values are Spatial Binning=2x2 and Temporal Binning=4, with total binning of 16. Data Type field allows changing of the size of the saved pixel records. This filed should be kept on USHORT.

Continuous Period and Cycles. Product of these fields defines duration of one run (shown in the Continuous-Time field). It determines how many frames the program will acquire. These values are computed from Continuous-Time and InterFrame Time (defined in the Hardware dialog) and reported in the Number of Frames fields.

Experiment Number and Run. These fields identify experimental runs and used to generate file names together with the Date fields. The program does their bookkeeping. The experimenter can modify these fields if runs should be rerun or to skip certain values. This is rarely done.

All other fields are commentaries. Their values are not important for data acquisition and saving.

Running ContImage in the Experiment mode

Pressing “Run” button (see Figures 8 and 10) starts frame acquisition and saving in the Experiment mode. Other buttons of the Experiment tool bar allow to:

Pause experiment. Pausing experiment running in the continuous mode is a bad practice since the continuity of the time series is lost.

Stop experiment. This button stops experiment and keeps the acquired data files.

Abort experiment. This button stops experiment and removes the acquired data files.

Display frames. It is highly recommended to disable display of the frames by un-checking this box while running ContImage in the Experiment mode to reduce CPU time utilization.

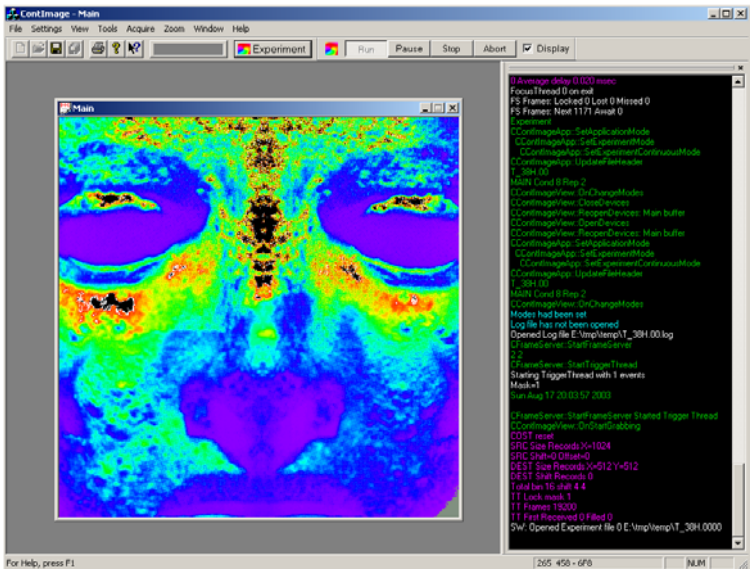


Figure 10. *ContImage running in Experiment mode. The blackened pixels indicate saturation and must be avoided. The "Display" check-box should be checked after beginning of the run to reduce CPU time utilization. Note that the "Run" button stays pressed for duration of the acquisition. It will go to the un-pressed state after end of the acquisition.*

Recommended sequence of steps for running typical experiment

1 Create directory for saving data files on a local or remote computer.
2 Start ContImage.
3 Open the Experiment dialog and modify the fields appropriately.
4 Close the Experiment dialog (by pressing OK to keep the changes).
5 Save the new configuration if needed for next invocations of ContImage by pressing File ⇨ Save Settings
6 Select the Focus mode (Main tool bar).
7 Select the Fast Focus mode (Focus dialog box).
8 Start the Focus mode (by pressing Run button).
9 Find ROI on the cortex and focus the camera (green light used for illumination).
10 Stop Focus mode (by pressing Stop button).
11 Switch to the ROI Focus mode (Focus dialog box).
12 Start Focus mode and select sub-ROIs if needed.
13 Save the green images (by pressing Save button). It is recommended to Stop Focus mode first and then save the images.
14 Defocus by lowering the camera.
15 Optional: Take intermediate green images while defocusing.
16 Switch to the Exposure Focus mode (by pressing Exposure button of the Main tool bar).
17 Switch to the Fast Focus view mode (Focus dialog box).

18 Start the Focus mode (by pressing Run button) and adjust illumination appropriately by moving the light guides around (red light used for illumination).
19 Switch to the Experiment Focus view mode (Focus dialog box).
20 Adjust the level of illumination (Acopian power supply). White pixels should be present and no black pixels.
21 Stop the Focus mode (by pressing Stop button).
22 Switch to the Experiment mode (by pressing Experiment button on the Main tool bar).
23 Start stimulation program.
24 Wait for a cycle or two.
25 Hit the Run button to start experiment.
26 Uncheck the Display check box.

Appendix

File naming scheme

Green – g_36e.0000
Exposure – e_36e.0000
Data file – t_36e.0000

Fields in file name (after the underscore, the period is not counted):
1 – year (2003 = 3) (possible values: 0, 1, 2,…, 8, 9, a, b, c, d,…, z)
2 – month (June = 6) (possible values: 1, 2, 3, 4, 5, 6, 7, 8, 9, a, b, c)
3 – day (14 = e) (possible values: 1, 2, 3,…, 8, 9, a, b, c, d,…, u)
4 – Experiment number (possible values: 0,1,2,…,8,9,a,b,c,d,…,z)
5 – Run number (possible values: 0,1,2,…,8,9,a,b,c,d,…,z)
6 – ROI number (possible values: 0,1,2,…,8,9,a,b,c,d,…,t; up to 31 sub-ROIs)
The main ROI has index 0.
7. – Identifies file number in a series of saved files.
8 and 9 – Added and augmented to prevent overriding existing data.