# **Supplementary** **Information**

The supporting information contains 23 pages, one AutoIt computer script, one MATLAB script, one Table, two Figures, and available as an attachment is 1 .csv file.

# **1 Experimental Section**

## **1.1 AutoItcomputerscript**

#cs Start of comment

This script is based on timed mouse clicks and keyboard entries.

In order that the script works, the user needs to have a monitor with the same screen resolution as the one used for our case (1366 x 768 pixels).

If not, the parameters passed to the functions regarding positioning of mouse clicks will need to be changed in order that the script works properly.

This script deals entirely with the software controlling the AIRICA by means of automating its operation.

It consists of a large infinite loop (While 1 … WEND) that has two main parts: the first part is optimizes sampling and analysis for concentration measurements, while the second part optimizes them for isotopic measurement.

Learn more about AutoIt for Laboratory Automation from Practical Laboratory Automation Made easy with AutoIt by Matheus Carvalho ISBN: 978-3-527-34158-0.

#ce End of comment

opt("Wintitlematchmode",1)

While 1

WinMove("AIRICA measurement","",0,0)

WinActivate("AIRICA measurement")

;\*\*\*\*\*\*\*\*\*\*\*\*

;Start of first part of the script

;\*\*\*\*\*\*\*\*\*\*\*\*

;clicking on edit sample list

MouseClick("left",50,180)

Sleep(1000)

;adding sample name

MouseClick("left",440,508)

Sleep(1000)

Send("{BACKSPACE 50}{DEL 50}")

Send("Sample")

Send(@YDAY&@HOUR&@MIN)

Sleep(500)

MouseClick("left",520,508)

Sleep(500)

MouseClick("left",645,508)

Sleep(1000)

;clicking on edit calculation mode

MouseClick("left",200,255)

Sleep(1000)

MouseClick("left",300,265)

Send("{BACKSPACE 5}{DEL 5}")

Send("2")

Sleep(1000)

MouseClick("left",320,305)

Sleep(1000)

Send("{UP 10}")

Send("{ENTER}")

MouseClick("left",475,305)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;clicking on edit extraction parameters

MouseClick("left",175,325)

Sleep(1000)

MouseClick("left",645,175)

Send("{BACKSPACE 5}{DEL 5}")

Send("210")

Sleep(500)

MouseClick("left",645,200)

Send("{BACKSPACE 5}{DEL 5}")

Send("50")

Sleep(500)

MouseClick("left",645,225)

Send("{BACKSPACE 5}{DEL 5}")

Send("60")

Sleep(500)

MouseClick("left",645,250)

Send("{BACKSPACE 5}{DEL 5}")

Send("0")

Sleep(500)

MouseClick("left",645,370)

Send("{BACKSPACE 5}{DEL 5}")

Send("80")

Sleep(500)

MouseClick("left",645,418)

Send("{BACKSPACE 5}{DEL 5}")

Send("2")

Sleep(500)

MouseClick("left",645,440)

Send("{DOWN 5}{UP}")

Send("{ENTER}")

MouseClick("left",635,535)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;click on set flow parameter

MouseClick("left",910,85)

Sleep(1000)

MouseClick("left",630,325)

Send("{BACKSPACE 5}{DEL 5}")

Send("300")

MouseClick("left",630,388)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;click on set blank

MouseClick("left",560,85)

Sleep(1000)

MouseClick("left",500,420)

Send("{BACKSPACE 10}{DEL 10}")

Send("2")

MouseClick("left",595,430)

Sleep(1000)

;clicking on sample to start measurement

MouseClick("left",224,134)

Sleep(1000)

MouseClick("left",95,95)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;monitoring a pixel in order to deal with different analysis times, if necessary

Sleep(10\*1000)

$TargetPixelColor = PixelGetColor(268,63)

While $TargetPixelColor <> 6684444

Sleep(5000)

$TargetPixelColor = PixelGetColor(268,63)

WEnd

Sleep(20\*1000)

;\*\*\*\*\*\*\*\*\*\*\*\*

;Start of second part of the script

;\*\*\*\*\*\*\*\*\*\*\*\*

;clicking on edit sample list

WinMove("AIRICA measurement","",0,0)

WinActivate("AIRICA measurement")

MouseClick("left",50,180)

Sleep(1000)

;adding sample name

MouseClick("left",440,508)

Sleep(1000)

Send("{BACKSPACE 50}{DEL 50}")

Send("Sample")

Send(@YDAY&@HOUR&@MIN)

Sleep(500)

MouseClick("left",520,508)

Sleep(500)

MouseClick("left",645,508)

Sleep(1000)

;clicking on edit calculation mode

MouseClick("left",200,255)

Sleep(1000)

MouseClick("left",300,265)

Send("{BACKSPACE 5}{DEL 5}")

Send("1")

Sleep(1000)

MouseClick("left",320,305)

Sleep(1000)

Send("{UP 10}")

Send("{ENTER}")

MouseClick("left",475,305)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;clicking on edit extraction parameters

MouseClick("left",175,325)

Sleep(1000)

MouseClick("left",645,175)

Send("{BACKSPACE 5}{DEL 5}")

Send("380")

Sleep(500)

MouseClick("left",645,200)

Send("{BACKSPACE 5}{DEL 5}")

Send("25")

Sleep(500)

MouseClick("left",645,225)

Send("{BACKSPACE 5}{DEL 5}")

Send("45")

Sleep(500)

MouseClick("left",645,250)

Send("{BACKSPACE 5}{DEL 5}")

Send("0")

Sleep(500)

MouseClick("left",645,370)

Send("{BACKSPACE 5}{DEL 5}")

Send("15")

Sleep(500)

MouseClick("left",645,418)

Send("{BACKSPACE 5}{DEL 5}")

Send("1")

Sleep(500)

MouseClick("left",645,440)

Send("{UP 6}")

Send("{ENTER}")

MouseClick("left",635,535)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;click on set flow parameter

MouseClick("left",910,85)

Sleep(1000)

MouseClick("left",630,325)

Send("{BACKSPACE 5}{DEL 5}")

Send("70")

Sleep(1000)

MouseClick("left",630,388)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

;click on set blank

MouseClick("left",560,85)

Sleep(1000)

MouseClick("left",500,420)

Send("{BACKSPACE 10}{DEL 10}")

Send("200")

Sleep(1000)

MouseClick("left",595,430)

Sleep(1000)

;clicking on sample to start measurement

MouseClick("left",224,134)

Sleep(1000)

MouseClick("left",95,95)

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

Send("{ENTER}")

Sleep(1000)

Sleep(10\*1000)

$TargetPixelColor = PixelGetColor(268,63)

While $TargetPixelColor <> 6684444

Sleep(5000)

$TargetPixelColor = PixelGetColor(268,63)

WEnd

MouseClick("left",938,124)

Sleep(1000)

MouseClick("left",670,405)

Send("{BACKSPACE 10}{DEL 10}")

Send("300")

Sleep(8000)

MouseClick("left",765,400)

Sleep(1000)

Sleep(50\*1000)

WEnd

## **1.2 Matlabscript**

%Matlab Script for processing CRDS data from PICARRO (csv export) 01/08/2016

%Inquiries please direct to K.G. Schulz (kai.schulz@scu.edu.au)

%1) Remove headers from PICARRO output csv (see PICARRO\_out.csv for details)

%2) Replace comma by space in PICARRO output csv

%3) Remove occasional 'Z' in last line of PICARRO output csv

%4) PICARRO output csv has to start below peak cut-off def., i.e. pCO2 below 400

%5) Remove any prolonged portions above the cut-off def., which

%are not associate to peaks from seawater injections

%(e.g. measurements of laboratory air). Otherwise those parts will be

%identified as peaks and inflate the matrix.

%6) Delta13C\_DIC values for the large CRDS injections and

%corresponding times are stored in a matrix called 'FinalOutput'

clear all

close all

fread='PICARRO\_out.csv'; %name of PICARRO input csv in same folder as

%this script

%means of carrier gas (instrument air) C12 and C13 concentrations

%and isotopic signature determined from several hours of continuous measurements

carriergas12=285.8;

carriergas13=3.181;

carriergasdelta=-10.09;

%BE CAREFUL WHEN MAKING CHANGES BEYOND THIS POINT

%----------------------------------------------------------

[DATE TIME AMPM FRAC\_DAYS\_SINCE\_JAN1 FRAC\_HRS\_SINCE\_JAN1 JULIAN\_DAYS EPOCH\_TIME ALARM\_STATUS INST\_STATUS CavityPressure CavityTemp WarmBoxTemp EtalonTemp DasTemp MPVPosition solenoid\_valves OutletValve MeasurementMode species HP\_12CH4 HP\_12CH4\_dry HP\_13CH4 HR\_13CH4 Delta\_iCH4\_Raw HP\_Delta\_iCH4\_Raw HP\_Delta\_iCH4\_30s HP\_Delta\_iCH4\_2min HP\_Delta\_iCH4\_5min HR\_12CH4 HR\_12CH4\_dry HR\_Delta\_iCH4\_Raw HR\_Delta\_iCH4\_30s HR\_Delta\_iCH4\_2min HR\_Delta\_iCH4\_5min ChemDetect H2O X12CO2 X12CO2\_dry X13CO2 Delta\_Raw\_iCO2 Delta\_30s\_iCO2 Delta\_2min\_iCO2 Delta\_5min\_iCO2 peak87\_baseave\_spec peak88\_baseave peakheight\_5 peak0\_spec ch4\_splinemax peak30\_spec peak\_30]=textread(fread,'%s %s %s %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f %f');

fclose('all')

%normalize time axis to beginning of experiment on Jan 19th 12:00

time=JULIAN\_DAYS-19.5;

%find all 12C data above 400ppm and the start and end point for

%each peak

pfsa=find(X12CO2>400);

dpfsa=find(diff(pfsa)>10);

%Compiling peak area data in a single matrix each

peaks12=ones(max(diff(dpfsa)),round(length(dpfsa)))\*NaN;

peaks13=ones(max(diff(dpfsa)),round(length(dpfsa)))\*NaN;

deltaC=ones(max(diff(dpfsa)),round(length(dpfsa)))\*NaN;

peakstime=ones(max(diff(dpfsa)),round(length(dpfsa)))\*NaN;

peaklength(1:(pfsa(dpfsa(1))-pfsa(1)+1),1)=(pfsa(dpfsa(1))-pfsa(1)+1);

peaks12(1:(pfsa(dpfsa(1))-pfsa(1)+1))=X12CO2([pfsa(1):pfsa(dpfsa(1))]);

peaks13(1:(pfsa(dpfsa(1))-pfsa(1)+1),1)=X13CO2([pfsa(1):pfsa(dpfsa(1))]);

deltaC(1:(pfsa(dpfsa(1))-pfsa(1)+1),1)=Delta\_Raw\_iCO2([pfsa(1):pfsa(dpfsa(1))]);

peakstime(1:(pfsa(dpfsa(1))-pfsa(1)+1))=time([pfsa(1):pfsa(dpfsa(1))]);

for i=2:length(dpfsa)

peaks12(1:(pfsa(dpfsa(i))-pfsa(dpfsa(i-1)+1)+1),i)= ...

X12CO2([pfsa(dpfsa(i-1)+1):pfsa(dpfsa(i))]);

peaks13(1:(pfsa(dpfsa(i))-pfsa(dpfsa(i-1)+1)+1),i)= ...

X13CO2([pfsa(dpfsa(i-1)+1):pfsa(dpfsa(i))]);

deltaC(1:(pfsa(dpfsa(i))-pfsa(dpfsa(i-1)+1)+1),i)= ...

Delta\_Raw\_iCO2([pfsa(dpfsa(i-1)+1):pfsa(dpfsa(i))]);

peakstime(1:(pfsa(dpfsa(i))-pfsa(dpfsa(i-1)+1)+1),i)= ...

time([pfsa(dpfsa(i-1)+1):pfsa(dpfsa(i))]);

peaklength(i)=(pfsa(dpfsa(i))-pfsa(dpfsa(i-1)+1)+1);

end

deltaC\_added=((peaks12+peaks13).\*deltaC-(carriergas12+ ...

carriergas13)\* ...

carriergasdelta)./(peaks12+peaks13-carriergas12- ...

carriergas13);

mean\_peakstime=nanmean(peakstime);

%filter for outliers, i.e. off by more than 2 std in comparison to

%mean

deltaC\_mean=nanmean(deltaC\_added);

deltaC\_std=nanstd(deltaC\_added);

deltaC\_added\_it1=deltaC\_added;

for i=1:length(dpfsa)

deltaC\_added\_it1(find( abs(deltaC\_mean(i)-deltaC\_added(:,i)) > abs(deltaC\_std(i).\*2)),i)=NaN;

end

deltaC\_mean\_it1=nanmean(deltaC\_added\_it1);

deltaC\_std\_it1=nanstd(deltaC\_added\_it1);

%%%%%%%%%%%%%%%%%%%%%second itteration

deltaC\_added\_it2=deltaC\_added\_it1;

for i=1:length(dpfsa)

deltaC\_added\_it2(find( abs(deltaC\_mean\_it1(i)-deltaC\_added\_it1(:,i)) > abs(deltaC\_std\_it1(i).\*2)),i)=NaN;

end

deltaC\_mean\_it2=nanmean(deltaC\_added\_it2);

deltaC\_std\_it2=nanstd(deltaC\_added\_it2);

%%%%%%%%%%%%%%%%%%%%%third itteration

deltaC\_added\_it3=deltaC\_added\_it2;

for i=1:length(dpfsa)

deltaC\_added\_it3(find( abs( deltaC\_mean\_it2(i)-deltaC\_added\_it2(:,i)) > abs(deltaC\_std\_it2(i).\*2)),i)=NaN;

end

deltaC\_mean\_it3=nanmean(deltaC\_added\_it3);

deltaC\_std\_it3=nanstd(deltaC\_added\_it3);

%%%%%%%%%%%%%%final itteration

deltaC\_added\_final=deltaC\_added;

deltaC\_mean\_final=deltaC\_mean;

deltaC\_std\_final=deltaC\_std;

for g=1:40

for i=1:length(dpfsa)

deltaC\_added\_final(find( abs(deltaC\_mean\_final(i)-deltaC\_added\_final(:,i)) > abs(deltaC\_std\_final(i).\*2)),i)=NaN;

end

deltaC\_mean\_final=nanmean(deltaC\_added\_final);

deltaC\_std\_final=nanstd(deltaC\_added\_final);

end

%distinguish between small peaks for DIC measurements and large for

%d13C\_DIC

large=find(peaklength>170&peaklength<450);

small=find(peaklength<150&peaklength>25);

%create matrix with data for large peaks only

C12\_pm\_large=peaks12(:,large);

delta\_pm\_large=deltaC(:,large);

time\_pm\_large=peakstime(:,large);

%final output

%measurement time and isotopic composition

FinalOutput=[mean\_peakstime(large)' deltaC\_mean\_final(large)']

%some data visualiziation

figure

axes('Position',[0.15 0.5 0.8 0.4])

plot(time, X12CO2,'k')

ylabel('^{12}CO\_2 (ppmv)')

xlabel('Time since start of the Experiment')

text(7.695,-400, 'Please note the two small peaks (seawater injections for determination of DIC)')

text(7.695,-490, 'followed by a large peak (injection for determination of carbon isotope signature)')

axis([7.695 7.84 0 1000])

figure

axes('Position',[0.15 0.5 0.8 0.4])

plot(time\_pm\_large,C12\_pm\_large)

ylabel('^{12}CO\_2 (ppmv)')

xlabel('Time since start of the Experiment')

text(7.695,-400, 'Large peaks as identified by the script, resulting from the slow seawater')

text(7.695,-490, '\rm injection, and used for subsequent $\rm \delta^{13}C\_{DIC}$ calculations','Interpreter','latex')

axis([7.695 7.84 0 1000])

figure

axes('Position',[0.15 0.5 0.8 0.4])

plot(mean\_peakstime(large), deltaC\_mean\_final(large),'ko','MarkerFaceColor','k')

ylabel('\rm \delta^{13}C\_{DIC} (^{\fontsize{7}o}/{\fontsize{7}oo})','Interpreter','tex')

xlabel('Time since start of the Experiment')

text(7.695,-3.4, 'Calculated $\rm \delta^{13}C\_{DIC}$, using the large paeks','Interpreter','latex')

axis([7.695 7.84 -3 -2])

end

## **1.3**



**Figure S1.** Typical output from the CRDS showing 3 measurements cycles for 13CO2 (top) and 12CO2 (bottom). An individual measurement cycle consists of the AIRICA system acidifying 3 aliquots of water. The first is a rinse, the second used to determine DIC concentration, and the third to determine δ13C-DIC.

# **2 Results and Discussion**

**2.1 Table S1.** δ13C values – IRMS (‰)

|  |  |  |
| --- | --- | --- |
|  | δ13C – IRMS (‰) | δ13C DIC – AIRICA-CRDS (‰) |
| Carbonate Standard 1 (Na2CO3) | -3.2 ± 0.1 (n = 3) |  |
| Standard 1 (~500 μmol) |  | -3.47 ± 0.63 (n = 7) |
| Standard 1 (~1000 μmol) |  | -3.77 ± 0.17 (n = 6) |
| Standard 1 (~1900 μmol) |  | -3.68 ± 0.16 (n = 57) |
| Standard 1 (~2800 μmol) |  | -3.60 ± 0.11 (n = 6) |
| Standard 1 (~3600 μmol) |  | -3.58 ± 0.07 (n = 8) |
| Carbonate Standard 2 (K2CO3) | -26.8 ± 0.1 (n = 3) |  |
| Standard 2 (~2000 μmol) |  | -26.67 ± 0.17 (n = 7) |

**2.2**

****

**Figure S2.** Regressions of 1/DIC concentration vs. δ13C-DIC values (Keeling plots). Letters correspond with the linear increase in DIC concentrations shown in Figure 4.