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Nova course 10.-14.6.2019
Viikki Science Park, Helsinki

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# Learning diary: Image-based phenotyping

**Task**
*Choose two presentation every day (Monday-Friday) from which you will write a learning diary.*

*Summarize:*

1. *The main points of the lecture*
2. *What (for you) important ideas you learned*

*The length of one diary is approximately one sheet (spacing 1.5, Times New Roman font size 12).*

*Speakers have provided their slides that will help you to review the lectures and also some literature references have been provided.*

*Return the learning diaries by e-mail to* *kristiina.himanen@helsinki.fi* *as soon as possible, but the latest by the end of July 2019.*

## Monday-Tuesday schedule

|  |  |  |
| --- | --- | --- |
|  | **Monday June 10 Phenomics** | **Tuesday June 11 Imaging stress** |
| 8-9 | **Breakfast together Viikki\*** | **Breakfast together Viikki\*** |
| 09.00 | Course introduction Teachers introduction (all teachers) K111/C1 | EnBlightMe – detecting plant diseases in the field(Erik Alexandersson) |
| 10.30 | break | break |
| 11.00 | Low cost phenotyping set ups and Specalyzer(Aakash Chawade) | Chlorophyll fluorescence for plant stress phenotyping (Mirko Pavicic) |
| 12.00 | Lunch break | Lunch break |
| 13.00 | Introduction of the NaPPI facilities(Katriina Mouhu) | Hyper- and multispectral imaging for health anddisease (Markku Keinänen) |
| 14.00 | Workshop 1. in 4 groups Two groups:1. Arabidopsis under stress -> Metabolomics sampling
2. Large plant phenotyping Two groups:
3. TBD
4. Specalyzer
 | Workshop 2. in 4 groups Two groups:1. Arabidopsis under stress -> Metabolomics sampling
2. Large plant phenotyping Two groups:
3. TBD
4. Specalyzer
 |
| 15.00 |
|
| 16.00 | Workshop 1 sum up (meeting rooms B145, 117) | Workshop 2 sum up (meeting rooms B145, 117) |
|
| 17.30 Evening: | **Students introduction over pizza dinner** | **Preparation of journal club presentations and questions in assigned groups** |
|

## Wednesday-Friday schedule

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Wednesday June 12 Metabolomics** | **Thursday June 13 Field Phenomics** | **Friday June 14 Integrated omics** |
| 8-9 | **Breakfast together Viikki\*** | **Breakfast together Viikki\*** | **Breakfast together Viikki\*** |
| 09.00 | Journal Club: Student presentations of the pre- assigned literature in groups (all teachers)break | Field phenomics (Morten Lillemo)break | Gene ontology lecture (Erik Alexandersson) |
| 10.30 | break |
| 11.00 | Introduction to Metabolomics(Markku Keinänen) | Public privatepartnerships for breeding (Magnus Göransson Agriculture University of Iceland) | GoMapMan and MapMan for metabolomics analysis (Špela Baebler) |
| 12.00 | Lunch break | Lunch break | Lunch break |
| 13.00 | Workshop 3.Metabolomics methods, visit to metabolomics lab and introduction to data analysis(Markku Keinänen, Nina Sipari) | Workshop 4.Processing and analysis of drone images(Ingunn Burud and Sahameh Shafiee) | Workshop 5. Metabolomics with GoMapMan (EA, SB) |
| 14.00 |
| 15.00 | Workshop 3 sum up |
| Course wrap up and evaluationDeparture |
| 16.00 |
| **Departure @ 17.00 Social event on Suomenlinna island** -starts at 18.35 |
| 17.30 Evening: |
| **Course dinner in Viikki area** |

Sunday Travel Joensuu-Helsinki

We took a train 12:13-14:40 to Helsinki. On the way, we were reading the papers and downloading the software.

JGroup3 (Journal Group3) will present

* Sugar beet worm / Aerial imaging paper is quite easy to understand.
	+ Problem
	+ Afrin will write Materials&Methods section of the presentation

JGroup3 will make questions for JGroup6

* Analysing phenotypes paper is hard to understand.
	+ Less pictures
	+ Lagas and Anne Mari agreed about this on the breakfast.

Monday 09 Kristiina’s introduction. We reached C1-classroom, which will be our base for the course. People introduced themselves:

* Kristiina Himanen, coordinator of the course
* Markku Keinanen, UEF, knows phenotyping
	+ Mats Gronlund, knows the tools well (Markku’s student)
	+ girl, very good with…, (Markku’s student)
	+ has developed MS-phenotyper (mass spectrometer imaging)
* ,from Norway
	+ , Norwegian lady
	+ , Middle-Eastern lady

How does the cart navigate?

* Going around in x and y?
* Would it be more easy to use center pivot irrigation? (Have an attached rail and move the camera along it radially.) In Qatar they are irrigating the desert with such rotating irrigation beams. <https://en.wikipedia.org/wiki/Center_pivot_irrigation>
* Pekka Vienonen’s master’s thesis <http://integraali.com/gradu/1997_vienonen.pdf>

“Producing an image of a cylinder, from a video, where it is rotating”

11 Aakash Chawade, SLU, Sweden

* Low-level phenotyping, people will walk in the field and study the pnenotypes
* Akash was telling about the phenotyping cart, which goes around in the field and takeHow does the cart navigate itself?

Aakash.chawade@slu.se

limnotic

beam going here and there like in a 3d printer

* students doing 3d printing emigrating to phenotyping?
* Camera moving with cable
* Robot camera
* Lawn mover camera
* Sheep eating grass camera

13 Kristiina Himanen, NaPPI network introduction

* NaPPI in general
* Markku Keinanen has stuff in UEF, Joensuu, will give a talk
* In Helsinki, other stuff, complementing
* Plants in the conveyor belt
* Plants in growth chamber
* anything for school children, plant growing competition?

=> Workshop 1.2 Phenotyping in NaPPI

Katriina Mouhu

14 Arabidopsis NaPPI

* Mirko Pavicic took us
* arabidopsis plants are in a growth-and-imaging room and are moved with conveyor belts
* after dark adaptation, when chlorophyl is measured, at first the plant is shiny and absorbing the light
	+ plant starts to resist the light in different ways
		- phosforesence
		- reflecting
* While taking photo, the light will reflect from the surrounding tent. To eliminate this, it is possible to send light pulses and record the pulse from the plant – before the pulse comes from the tent walls!
	+ This is similar to bats. Bats use echo to navigate. Bats can disconnect their hearing bones. In this way, they don’t hear themselves screaming, but will only hear the echo. Very good thing for the sensitive ears of a bat!

<https://www.thoughtco.com/how-bat-echolocation-works-4152159>

14:30 Large plant phenotyping

* Katriina Molsa took us to a greenhouse, where potatoes are phenotyped
* There were around 300 potato plants
* Plants go: dark adaptation, imaging etc. a plant stays 30mins in the tent
	+ need to do imaging in batches
	+ need to do imaging in specific time of the day, when there is less variation in the daylight: morning 2-6am is good, winter is good
	+ if there is system failure, researchers are emailed and will come to fix it
		- better to stay near the green room, in case there is a system failure
* Plants are watered 2-3 times per day

15-17 doing stuff

18 student presentations

* it was interesting to hear about people
* some people had short introduction, some people had more
* Dylan had a map of Great Britain and a map of his campus, it was nice
* old man was studying how plants grow in long-war-torn areas, but doesn’t have funding now – I guess the funding countries don’t want to support the research
* pizza was late
* people decided to have all the presentations before eating the pizza – not a good decision!
	+ pizza got cool
	+ it was tiring to listen

19 getting back to apartment to Vuolukiventie

* I found the walking pathway through the small roads

00 I was studying

09 Markku Keinanen – hyperspectral imaging

* Markku was very nicely demonstrating how by hyperspectral imaging you can find some plant diseases
* archityping is a data-based, automatic algorithm, which will find architypes
* plant = 0.8 healthy + 0.12 drought + 0.6 mold = 0.6 something
* red lamp + filter + gray scale camera = 200€ imaging system
* Sm4rtlab in Joensuu has a system to work with plants in browser

Somebody asked about the difference of NIR (near infrared) and thermal imaging

* NIR is with 800nm
* thermal is with 8000nm

Markku explained the difference very nicely

11 Mirko Pavicic – digital imaging

* Mirko had very interesting slides, remember to ask the slides from Kristiina.
* Mirko will delete the things which he has not published yet.

[http://colorizer.org](http://colorizer.org/)

Fiji-software

Recalling how photosynthesis works

Photosystem I

* thylakoids: light-dependent reactions, light => ATP and NADPH
* stroma: light-independent reactions, ATP => sugar

Photosystem II

* ligth bouncing in pigments
* gets excited
* electron from water, water releases oxygen, hydrogen goes to plant

If there is too much light, then “violaxanthin de-epoxidase” will see the too many protons and change how the picment works

Chlorophyll a

The pulsed device

<http://www.hansatech-instruments.com/pulse-modulated-chlorophyll-fluorescence/>

Mirko showed beautiful video from NASA. This kind of videos make children interested about science!

Tue 13 Erik Alexandersson – EnBlightMe! - detecting plant diseases in the field

* Vinnova and IBM collaborating about potato imaging in Sweden

Potato:

* high value crop, 3rd most important food crop
* P. infestants causing potato late blight costs 7 billion USD per year
* High fungicide use in Sweden
* Early and accurate detection

Imaging

* RGB
* multispectral
* hyperspectral
* thermal
* chlorofyll-fluorescence
* 3d-sensors

Southern Sweden, cloudy areas

* How to reduce the use of fungicides?

Can infested plants be killed with

* rotating blades in drones
* lasers
* specific sprayed plant killer
* freezing
* burning
* denying irrigation

Projected image to a rectangle with Mobius transformation?

IBM Watson is a black box – is it nice to use a black box?

TensorFlow+Keras

invitation to classify 10000 images – how do collaboration invitations in conferences work?

Problem with mosaicing - compare with Pekka Vienonen thesis. **Juha-Matti sent email to Erik.**

Animal echolocation

Tue 14-15 Markku stuff in green room

Tue 15-16 Aakash Chawade and Alexander Koc – Specalyzer in [http://specalyzer.org](http://specalyzer.org/)

Article: <https://peerj.com/articles/5031/>

Specalyzer helps to analyze spectral measurements

* The website has data to practice with
* Data is in a text file, for example, “1-1.TRM”. It contains the intensity versus to wavelength.

" TRANS-> Wave:734.22nm Pix:1050 Val: 26.056 Time:10ms Avg:5 Sm:1 Sg:0 Tc:on SNAP Xt:3 Ch:1 Xtrig

344.50 2.1448E+000

345.00 1.7740E+000

345.50 1.3827E+000

* Attributes are in “attribute-data.tab”

filename nitrogen variety

1-1.TRM 140 1

1-2.TRM 140 1

1-3.TRM 180 1

* Data and attributes were uploaded to specalyzer
* First, visualizing
* It is seen that in beginning and end, the data is not good
* filtering 300,400,1000,1200
* We do PCA, and see outliers
* mouseover shows information of outlier
* removing outlier in filtering
* see the improved PCA
* start vegetation index visualization, which points a few good canditates. We were given a task to check which canditates would be meaningful

List of all indexes <https://www.indexdatabase.de/db/i.php>

Rep Li – is related to chlorophyl content!

Datt – is related to nitrogen content!

* The [Datt (1999)](https://www.sciencedirect.com/science/article/pii/S0168169919300614%22%20%5Cl%20%22b0060) [vegetation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/vegetation) index was least sensitive to variable water sufficiency.
* Source <https://www.sciencedirect.com/science/article/pii/S0168169919300614>
* Datt can be improved by eliminating something.
	+ This seems similar to developing Simpson’s rule from trapezoid rule and mid-point rule.

Vogelmann index is named after Vogelmann. This name is easy to remember: Vogelmann = bird man (German)

**Comments on the arrangements**

**Accommodation**

Sunday 20 Unihome Pihlajamaki

* room is nice, has nice kitchen
* Pihlis pizzeria has good pizza
* S-market open to 22
* bad smell in kitchen, because biowaste was rotting
* mattress is thin, in the morning back has pain

**Arrangements in university**

* Finding the path Vuolukiventie-university was a little difficult, but once found, it was easy to go.
* Monday 09 C1-classroom is oldfashioned, there are very less power outlets, should the university do some renovation?
* About the university
* for a guest, toilets were difficult to find
* for a guest, the doors closed quite early (summer time)
* some students had problems with eduroam

**Other**

Monday 08 Breakfast. We left from Vuolukiventie 1bS325 and headed to the breakfast. We took the path Vuolukiventie-Meripihkatie-Ronnbackantie-Latotie.

* At the breakfast, the restaurant is being held by Sodexo. Solevo is familiar to me from Savonia-UAS-Kuopio.
* Returning of trays is hidden behind the corner. There are no sign boards for the tray returning place.
* A cashier lady was saying “These phenology people should eat lunch at 10:30, not coffee”

Hangout place near Ladonlukko restaurant is very nice

* chalkboards are dear for my math husband
* beautiful yellow sofa next to window and plants

**Image here!**

My favorite place in the university

**Other studies**

I am doing two online courses in Jyvaskyla-UAS. I need to submit two course assignments

* One about sexual health education
* One about leadership. A good paper: <https://www.researchgate.net/publication/228535201_Servant_leadership_a_case_study_of_a_Canadian_health_care_innovator>

My husband, Juha-Matti, went on Wednesday to Turku, to meet a Japanese mathematician, who he will meet in July in Japan.

Thursday 11 Sahameh Shafiee NMBU – Aerial imaging

We were studying Pix4Dmapper

13 Magnus Goransson – Practical phenotyping in a small-scale breeding program in Iceland, Nordic PPP Project in Barley Pre-breeding Genetic resources – gene banks

Agricultural University of Iceland (AUI)

Magnus invited to go to Iceland and do field measurements.

In New Delhi, there are sandstorms, hurricanes etc. Temperature +48.

In Iceland, the pressure record 1048mbar was overtaken on 12.6.2019. Previous record from 1939.

In Iceland, there is drought now.

Punla grass doesn’t stop growing and will die (temperature, not day length).

<https://sesto.nordgen.org/sesto/index.php?thm=sesto>

<https://www.grin-global.org/>

<https://npgsweb.ars-grin.gov/gringlobal/search.aspx>

14 Sahameh Shafiee NMBU – Aerial imaging with Pix4D

* flying at 20m height can give GSD = Ground Sampling Distance, 1cm
* median of 10000 keypoints on image

What to do?

* Fly high, fly low?
* Edit data, fly again?

## Ideas after workshop

### Projected image made straight

Many times, the imaging is done with drone. If the land is flat, the drone will take an image, which is twisted in a projective way. There should be a simple mathematical way to make the image straight! The easiest idea is to take a linear transform.

**Example.** A line through points (a,b) and (c,d) is given by

y= h(x)\*b+(1-h(x))\*d, where h(x)= (c-x)/(c-a)

Easily, it is seen that it is an equation of a line and that h(a)=1 and h(c)=0 yielding y(a)=b and y(c)=d.

The resulting code is a modification of this idea.
A related master’s thesis: <http://integraali.com/gradu/1997_vienonen.pdf>

The code is given below and here: <http://integraali.com/octave/>
Perhaps OctaveOnline is the easiest option to run the code: <https://octave-online.net/>

|  |  |
| --- | --- |
|  | A close up of a sign  Description automatically generated |
| **Before transform:** A book is lying down on a table and a photo is taken. It is a very unusual angle to read a book! | **After transform:** The book is straight and easy to read. |

How the process looks in OctaveOnline:

|  |  |
| --- | --- |
| **A screenshot of a computer  Description automatically generated** | **A screenshot of a computer  Description automatically generated** |

**The code**

%coordinates of the vertices. No automatic process to do this yet.

a=52+7\*i;

b=139+128\*i;

c=10+95\*i;

d=87+217\*i;

%height-width-ratio

h=0.7;

%parameters for the process (number of steps/pixels)

N=200;

S=1;

Nh=round(N\*h);

t=linspace(0,1,N);

s=linspace(0,1,Nh);

%read the image to be processed

A=imread('sbook.jpeg');

B=floor(A/2);%producing an “aperture showing image”

%make a loop with parameters t and s.

for k=1:N

for l=1:Nh

p=a\*(1-t(k))\*(1-s(l))+b\*t(k)\*(1-s(l))+c\*(1-t(k))\*s(l)+d\*t(k)\*s(l);

%”pick-up-point” p is an weighted average of the points a,b,c,d

P=p+2+2\*i;

x=floor(real(P));

y=floor(imag(P));

fx=real(P)-x;

fy=imag(P)-y;

f(k,l,:)=A(x,y,:)\*(1-fx)\*(1-fy)+A(x+1,y,:)\*fx\*(1-fy)+A(x,y+1,:)\*(1-fx)\*fy+A(x+1,y+1,:)\*fx\*fy;

B(x,y,:)=A(x,y,:);

end

end

imshow(f)%result

imshow(B)%aperture

## Videos

Juha-Matti took some videos during the event. They are listed here.

**Note!** The videos are “unlisted” in YouTube. Therefore, you can access the videos only if you know the specific link.

**Video 1: Nova course 2019. Computer is processing the Arabidopsis plants.** <https://www.youtube.com/watch?v=ONEPHfzC2ek>

**Video 2: Nova course 2019. Arabidopsis imaging**. <https://www.youtube.com/watch?v=IdJhQ97EM0g>

**Video 3: Nova course 2019. Potato going to imaging**. <https://www.youtube.com/watch?v=f1tRkQ2eEfs>

**Video 4: Nova course 2019. Potato going to imaging 2**. <https://www.youtube.com/watch?v=XH2hCL4U2zg>

**Video 5: Unihome door bang**. <https://www.youtube.com/watch?v=y47DquK8iPQ>

Loppukevennys /the final relief. While going to the kitchen, it is a good idea to keep the door in such a way, that it will not lock. But the door spring is so tense that it will pull the door shut very fast! This results in a huge bang!