



## **Objectives of the activity of WG 2 include:**

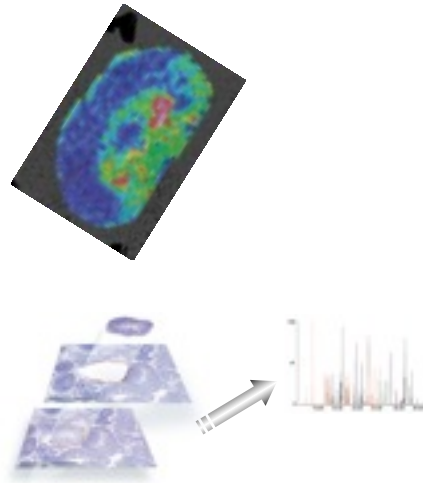
- 1)** Method optimization and standardization for the preparation of pure populations of all renal compartments from a) frozen and b) paraffin tissue sections following the use of Laser Capture Microdissection and imaging/profiling mass spectrometry.
- 2)** Method optimization for protein extraction, separation, quantification and detection of post-translational modifications of kidney proteins.
- 3)** Identification of existing infrastructure on nano-scale proteomics devices such as 2DE- lab on chip mini-devices and evaluation of their potential application for the analysis of starting material of limited amount, such as the protein extracts from LCM-isolated cells.



**Inform** EuroKUP community of the possibilities of tissue proteomics:

## Nafplion

- Isabelle Fournier: Maldi-imaging
- Theo Luider: LCM-proteomics



## Sheffield

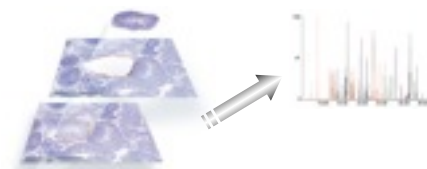
- Roz Banks: Proteomic analyses of formalin-fixed paraffin embedded tissue.
- Olivier Lapr evote: TOF-SIMS imaging of biological tissues.



**Train** EuroKUP community for tissue proteomics:

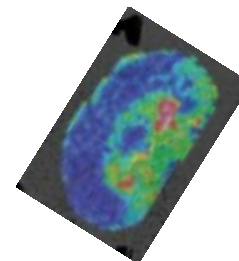
## Training School on Laser Capture Microdissection-Mass Spectrometry

**Where:** Rotterdam, NL  
**When:** May 25-27, 2009  
**Organiser:** Theo Luider



## A training school on MALDI Imaging of Kidney Tissue

**Where:** Helsinki, FI  
**When:** October 12-14, 2009  
**Organiser:** Marc Baumann



**Details later this morning.**



**Provide** tissue proteomics sample preparation protocols for EuroKUP community on EuroKUP website:

News About Contact Meetings Training Schools STSM Protocols

## Eurokup

Home

### protocols

View Edit Outline Track

This site summarizes Standard Operating Procedures (SOPs) developed and employed by EuroKUP members for biological specimen (urine-tissue) collection. These protocols have been widely employed for proteomics analysis and are therefore recommended to be used in the clinical setting

- **Collection of urine samples for peptidome (e.g. LC-MS or CE-MS) analysis**  
To achieve standardization in the clinical setting and facilitate data comparability, a detailed protocol for urine collection has been prepared and can be downloaded [here](#)
- **Rat and mouse kidneys preparation**  
This protocol for rat and mouse kidneys preparation was used in the Rotterdam training school. It can be downloaded [here](#)
- **Collection of urine samples for proteome analysis**  
A detailed protocol for urine collection employed in Prof Roz Bank's laboratory is described in the attached file. It can be downloaded [here](#)

joost schanstra

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#### news

- Training School on MALDI Imaging of Kidney Tissue: Open Call for Applications July 27, 2009
- EuroKUP / e-LICO May 14, 2009
- Presentations from the Nafplio Meeting May 8, 2009

+ ....Imaging protocols from Isabelle Fournier

Sheffield 2009



**Next ?**

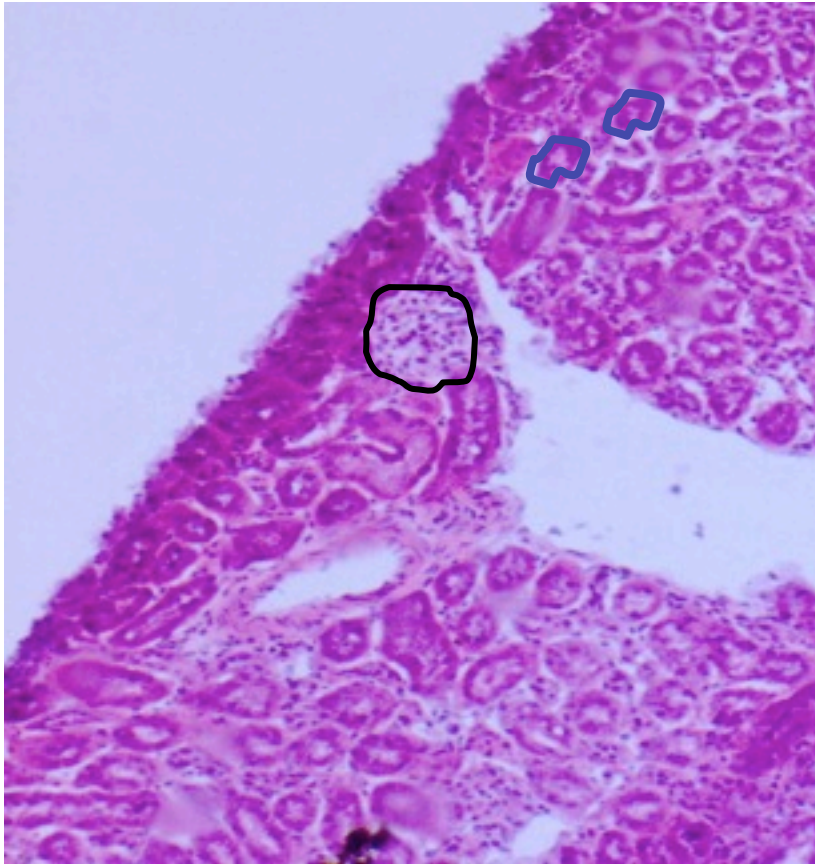


## Next:

1. Going beyond the “glomerulus *versus* tubule” ?
2. Store the data of the training schools on the EuroKUP website to start a database?
3. Training schools on quantification (SRM/MRM), post translational modifications, kidney structure/cell detection before proteomics analysis, other...?
4. Prepare a project of “cataloguing” kidney proteins (patho) physiological conditions?
5. A) Validation of protocols...”minimal SOPs”, B) more protocols
6. Topics for specialist presentations.

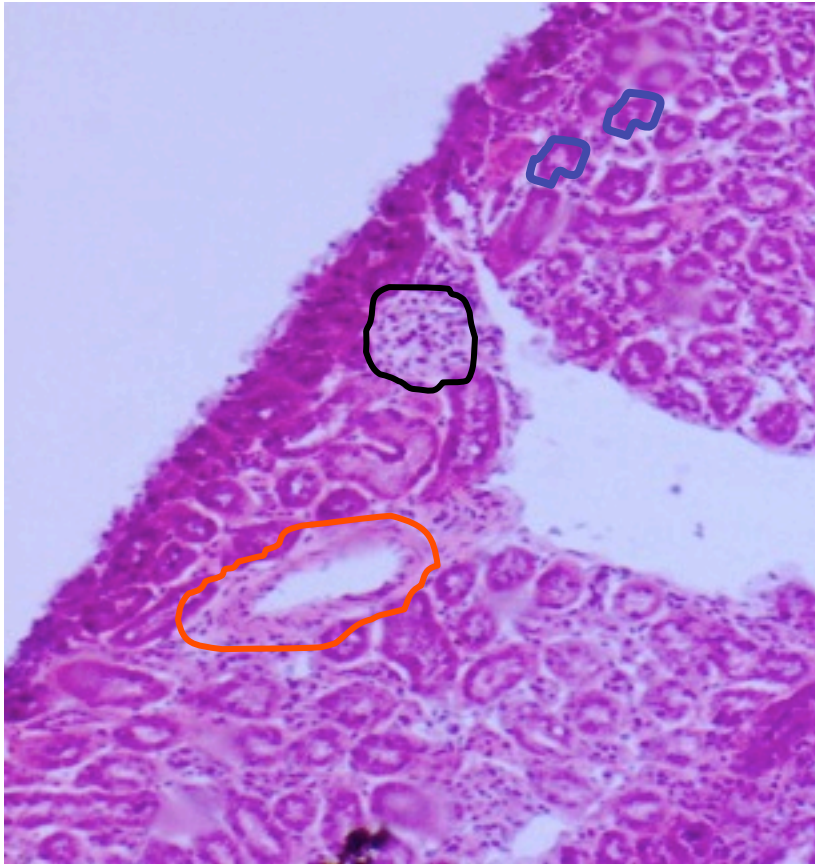


## 1) Going beyond the “glomerulus *versus* proximal tubules” ?



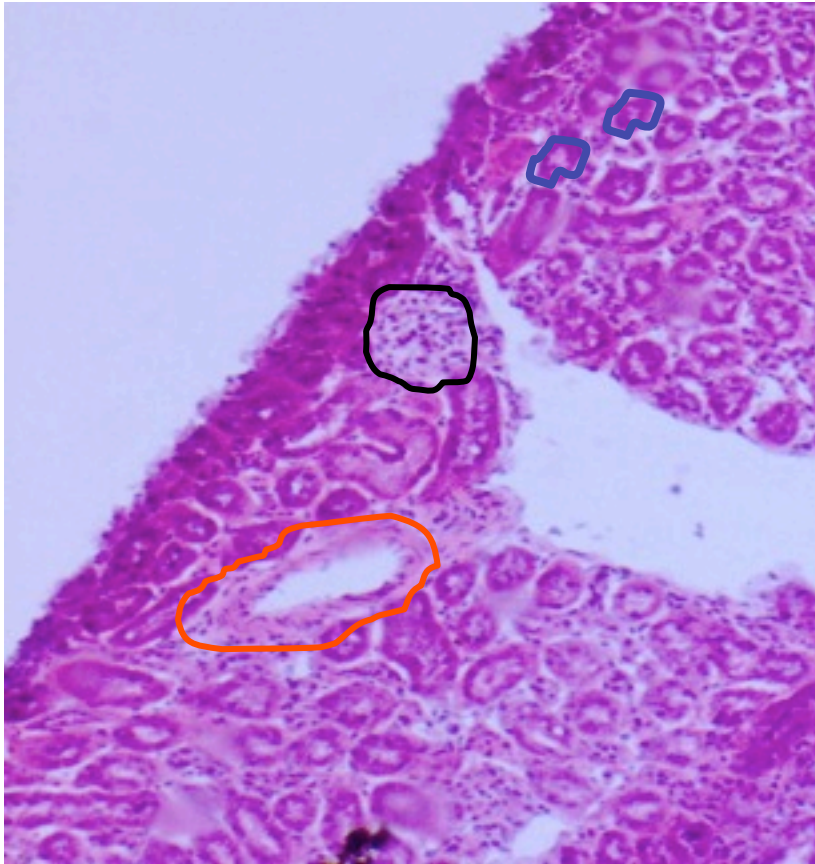


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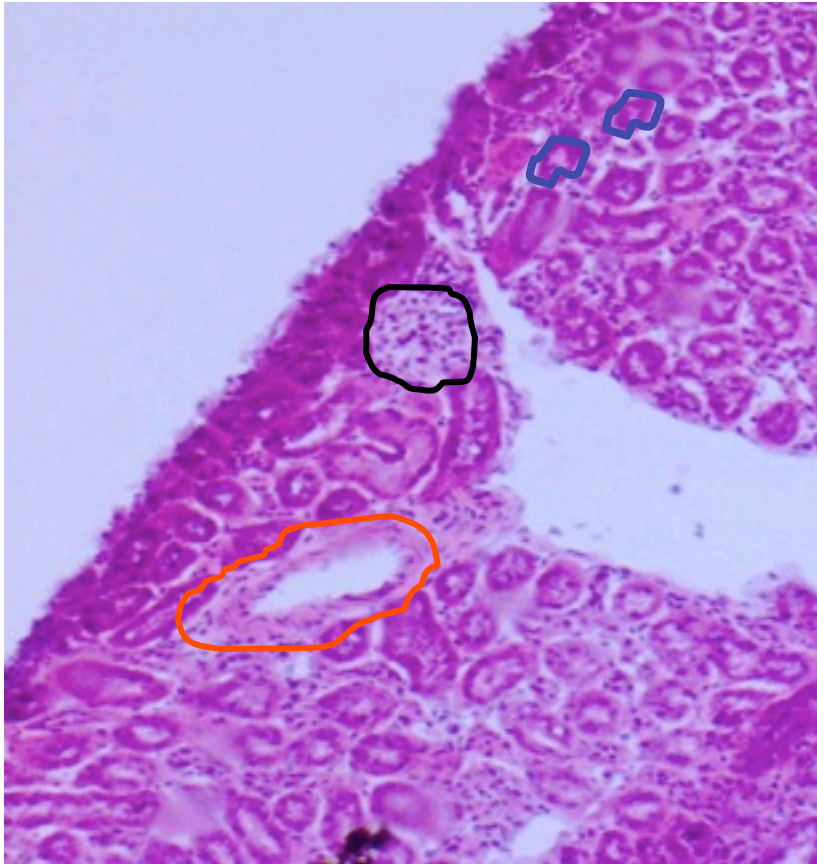
Identification of other renal structures

Involvement of renal pathologists

Immunoguided LCM



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### Identification of other renal structures

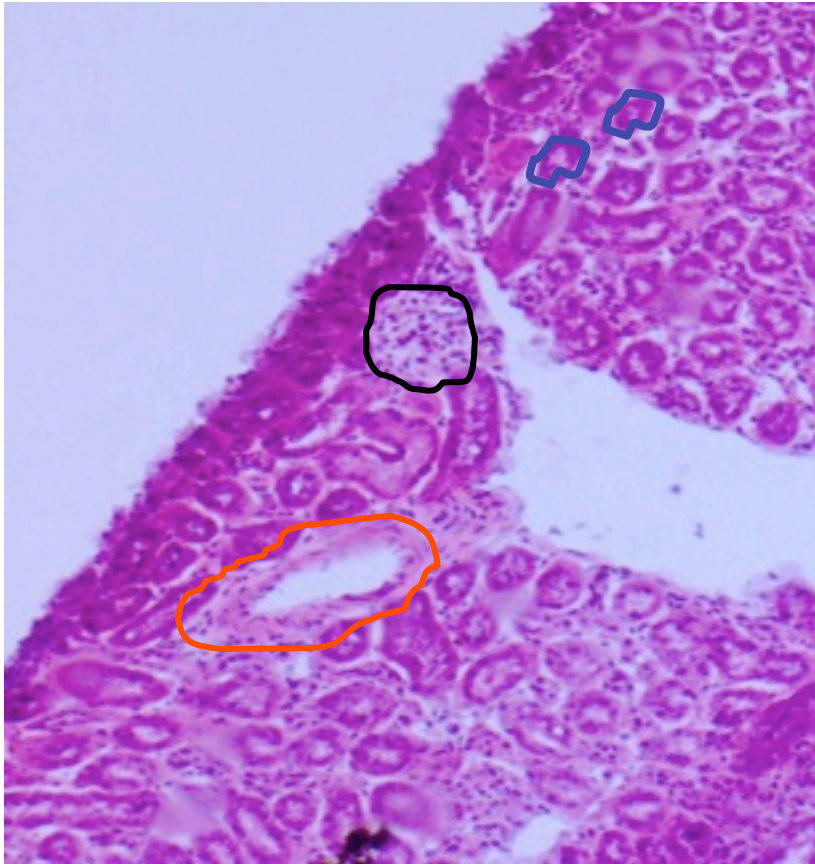
#### Involvement of renal pathologists

#### Immunoguided LCM

- for i.d. the nephron structure (transporters,....., etc).
- for renal cells, podocytes, mesangial cells, tubular cells (nephrin,....etc).



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### Identification of other renal structures

#### Involvement of renal pathologists

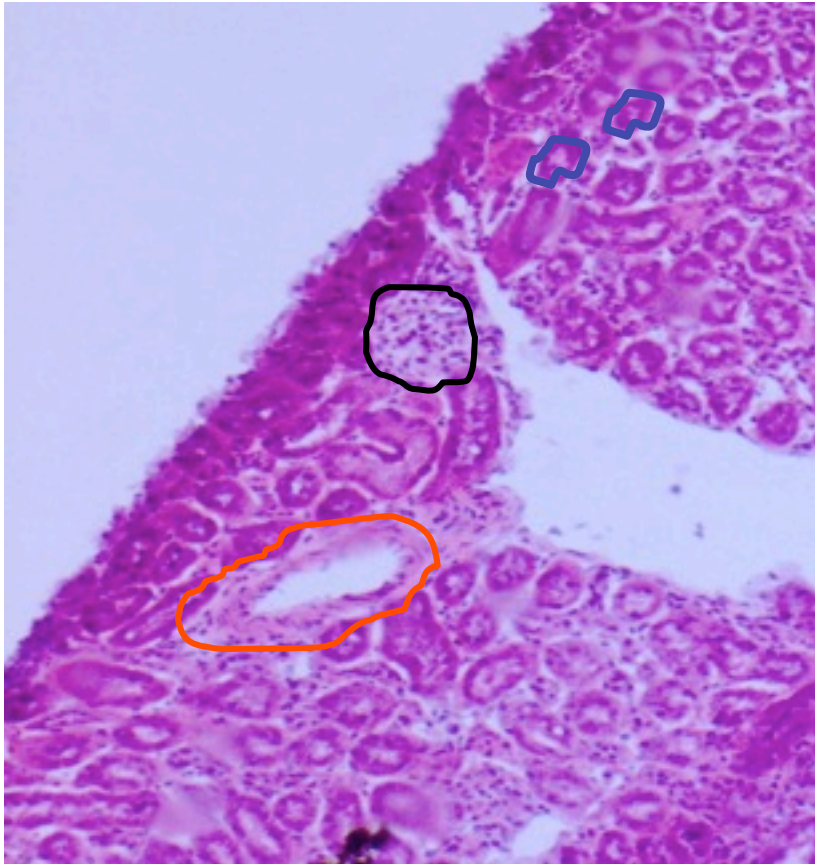
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*Establishment of a “consulting body” for defining areas in renal parenchyma and optimization of immunodetection of structures and cells before proteome analysis?*

**Volunteers/participants ??**

Sheffield 2009



2) Store the data of the training schools on the EuroKUP website to start a database? (→ **WG4**) ... for starters.



## EuroKUP-DB

- Urinary proteins (**WG 3**, different techniques).
- Glomerulus *versus* proximal tubule data rat and mouse (obtained by LCM/proteomics, Rotterdam).
- Imaging data (Helsinki).



## 3) Training schools on quantification, post translational modifications, other...?

- Imaging – Guenther Allmaier (2011).
- Identification of renal structures cells for LCM/Imaging...??



## 4) Prepare a project of “cataloguing” kidney tissue proteins under (patho) physiological conditions?

- Create a catalogue under physiological conditions?
- or ...choose a pathology.....
- Which tissue (animal, human).



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- Create a catalogue under physiological conditions?
  - or ...choose a pathology.....
  - Which tissue (animal, human).
- Can we use the data from the training school ? *Same tissue for all training schools?* (allow to combine the LMC and Imaging data?)



## 5A) Validation of protocols...”minimal SOPs”.

Protocol used for rat and mouse kidney preparation for LCM training school in Rotterdam (Joost Schanstra):



### Instructions



#### 1. Anaesthesia

Anaesthesia with pentobarbital. The quantities are determined by the weight of the animal and are described on the bottle/package.

#### 2. Perfusion

The animal should be immediately flushed with PBS. This is done by direct injection in the left ventricle of the heart by cutting (for pressure relief) the inferior vena cava (most accessible vein). Continue until the kidney “whitens”.

#### 3. Dissection

Dissection and decapsulation of the kidneys.

#### 4. Freezing

The kidneys should be frozen on Whatman paper in N<sub>2</sub>(l) vapour until the kidney hardens (gets really white). This avoids the kidney “cracking” and losing kidney structure.

#### 5. Packaging

Insert the frozen kidney, with Whatman paper, in appropriate sized tube (1.5 ml for mouse and Eppendorf and 15 ml Falcon for rat) and transfer to the N<sub>2</sub>(l).

#### 6. Storage and transport

Stored for long-term storage at -80°C. Transport on dry-ice.



## 5A) Validation of protocols... "minimal SOPs".

Protocol **proposed** for human biopsies in the CKD project.

(Hassan Dihazi and Joost Schanstra):

**Ideal** for downstream proteomics applications would be snap freezing in liquid nitrogen. This will allow to do almost anything afterwards.

Steps:

1) gloves all along the procedure.

2) take a cryo-vial (such as Nunc cryotube vials of 1 or 1.8 ml).

We can despatch these to the collaborating clinics, if necessary.

3) put a sticker with a code on the tube.

We can provide these stickers (resistant to liquid nitrogen and -80°C) with codes for each centre.

4) do not forget to link the sticker-code to your patient database.

Stickers with codes will be provided in double: one for the tubes and one for your file.

5) perform biopsy.

6) empty the needle in the tube.

7) close the tube tightly (important!).

In the most clinics there is no liquid nitrogen in the operation room. In our case (the research laboratories are integrated in the clinic) we take the biopsies from the operation room, we transport them on ice to the lab and put them in liquid nitrogen. If there is problem the process the biopsy because of the duration of the operation, we have a fridge in the operation room and the biopsies are stored at 4°C for a while before being transported to the lab.

8) drop the tube in liquid nitrogen. From now on, avoid thaw/freeze cycles!

9) take the frozen biopsies from the liquid nitrogen and Store at -80°C.

10) Transport on dry-ice once sufficient samples.

Problems/issues:

1) We have found out that liquid nitrogen is only rarely used for biopsies in routine clinical practice, at least in Toulouse.

So feedback is needed whether this type of protocol is feasible in all participating centres.

Concerning the liquid nitrogen step it can also be performed in the lab if the biopsies are transported on ice to the lab. The most important is to keep the samples cold after taken them from the patient.

2) Storage at -80°C.

Is this feasible in all participating centres?

For biopsies the -80 °C is necessary (at -20°C only for short time)



## **5B) More protocols on EuroKUP and Molmeth websites.**

- LCM/proteomics
- Paraffin embedded/proteomics

.....



## **6) Topics for “specialist presentations.”**

- **Pathologist presentation of the best ways to detect specialized kidney structures (experience, immunodetection, staining)**
- **What is going on in the urological field ?**
- **Other .....**