SCI3050
Advances in Biomedical Sciences

University College Maastricht

Period 2
2017 – 2018

Contents

General Information

Course Coordinators

Dr Matthew Baker (m.baker@maastrichtuniversity.nl)
Assistant Professor, Department of Complex Tissue Regeneration
MERLN Institute for Technology-Inspired Regenerative Medicine

Dr Vanessa LaPointe (v.lapointe@maastrichtuniversity.nl)
Assistant Professor, Department of Complex Tissue Regeneration
MERLN Institute for Technology-Inspired Regenerative Medicine

Lecturers

Dr Matthew Baker (m.baker@maastrichtuniversity.nl), Assistant Professor, MERLN
Dr Aurélie Carlier (a.carlier@maastrichtuniversity.nl), Assistant Professor, MERLN
Dr Shane Ellis (s.ellis@maastrichtuniversity.nl), Assistant Professor, M4I
Dr Tiffany Porta (t.porta@maastrichtuniversity.nl), Assistant Professor, M4I
Dr Raimond Ravelli (rbg.ravelli@maastrichtuniversity.nl), Assistant Professor, M4I
Dr Sabine van Rijt (s.vanrijt@maastrichtuniversity.nl), Assistant Professor, MERLN

Tutors

Dr Tiffany Porta (t.porta@maastrichtuniversity.nl), Assistant Professor, M4I
Alex Vasilevich (a.vasilevich@maastrichtuniversity.nl), PhD Candidate, MERLN (Task 8 workshop)

Communication

General announcements and schedule changes will be posted to EleUM, and personal communication will be made through your UM email address, so please check both regularly. We will aim to respond to your emails within 1–2 working days, but it is best to use the time we see each other in the lectures and tutorials to ask questions. The coordinators, lecturers and tutor are all based at Randwyck, so please email them if you wish to make an appointment.

Required course materials

This course highlights frontier discoveries that are generally not yet included in textbooks, so there is no textbook required for this course. Some course materials, such as scientific papers, will be available *via* EleUM.

Course Description & Objectives

The purpose of this course is to introduce students to recent breakthroughs in the biomedical sciences. Each lecture will be given by a different expert from one of the two new research institutes in FHML (MERLN and M4I). The course will highlight a broad range of topics from the biomedical sciences, including regenerative medicine, supramolecular chemistry, nanoscience, big data, mass spectrometry, and structural biology. The lectures and tasks will focus on the cutting-edge discoveries in these topics and how scientists hope to use the knowledge to cure diseases or solve other societal problems. Students will place the discoveries in a historical context and will learn about the activities taking place within the UM in these areas. There will be a visit to some of the laboratories of the researchers to see the science in action.

The overall objectives are:

- To gain insight into some frontier topics of the biomedical sciences
- To evaluate scientific breakthroughs as an educated society
- To apply knowledge from the natural sciences towards problems in society
- To increase an appreciation of the research being performed at UM in cutting-edge topics such as regenerative medicine, mass spectrometry, and electron microscopy
- To meet some young academic staff active in these fields and get to know them and their research

Course Procedures

Part of the course will be lecture-oriented. Due to nature of the content of this course, the lectures will be very important to your overall learning. Many of the topics we will cover cannot be found in a textbook. You are encouraged to print the slides in advance and use them as notes. During the tutorial groups, you will work on a task that will reinforce and supplement the concepts presented in the lectures. You will find that many of the tasks contain a mixture of technical information, a (bio)medical problem, and an ethical or societal issue. Your interdisciplinary background and liberal arts thinking will be put to the test in the midterm and final papers.

Some general rules and guidelines:

- Because of the wide variety of topics and the lack of pre-requisites, you may find that you lack a knowledge base for some of the lectures. Don't hesitate to ask the lecturer for some additional explanation of a challenging concept.
- You are encouraged to ask questions at any time during the lectures.
- Attendance to the lectures is highly recommended in view of the fact that the lecturers are experts in the topics and much of the information presented cannot be found elsewhere.
- As always, attendance to the tutorial group meetings is required and only a limited number of absences are permitted (*cf.* Rules and Regulations).

Assessment

Your learning will be assessed in the following ways:

- Midterm paper 25%
- Final paper 40%
- Group oral exam 25%
- Tutorial Participation 10%

Below is a short description of the expectations for each form of assessment. Please ask the Course Coordinators for more information if you have any questions. In case of a failing overall grade in this class, but a pass on attendance, a re-submission of the final paper will be allowed. However, your other assessment grades will remain unchanged.

Midterm paper

Your midterm assignment will be to choose a recent (2016–2017) news article about a scientific finding related to this course and write a ~1000-word piece about it after having investigated the claims. Your grade will reflect both the quality of the content and your writing. You should decide on the best format and content, but you may wish to consider/include:

- Why did this paper/finding end up in the news?
- What is the main finding and the evidence supporting it?
- A figure to explain the finding
- How accurate is the news report compared to the scientific paper?
- How might the journalist have improved their reporting?

Final paper

In the midterm, you were encouraged to judge the journalist, but now you have to be the journalist. Select one of the lecturers and interview them about their research. Write a ~1000-word piece about one (or more) of their findings and your perspective on them. Your grade will reflect both the quality of the content and your writing. You should decide on the best format and content, but you may wish to consider/include:

- Introduction to the field
- Highlight one or more important findings
- A figure to explain the finding(s)
- Perspective on the importance/relevance of the work (societal, economic, *etc*.)
- Insight into future directions

Grading of the midterm and final papers

You will receive a score based on the following elements:

- Writing (10%). Write clearly, concisely and convincingly with no typographical errors.
- Style (10%). Tell a story in your paper and bring the reader into the topic. Present your ideas in a logical way.
- Level (20%). Consider your target audience to be your peers, so write at a high level but explain advanced concepts.
- Research (25%). Do your homework on the topic and show your knowledge. Cite your sources.
- Non-research content (25%). Show your creativity when providing your opinion and perspective.
- Overall impression (10%). Make use of figures and captions for a top impression.

Final oral exam

To test your knowledge of the PBL tasks and overall course content, you will participate in an oral defence as a small group. You should expect to answer questions from the examiners related to the learning goals of the course. Team work will be important here, because you can choose who answers which question, but you will also be graded on your team work. We will speak more about this exam format during the course. It's common in science, but we understand you probably haven't done this during your studies. Your score will be based on the following elements:

- Knowledge (50%). Simply put, how correct are your answers.
- Convincingness (25%). Speak with clarity and confidence; defend your responses.
- Team work (25%). Do all team members answer an approximately equal number of questions? Do you appear to be working together?

Plagiarism

Plagiarism within the sciences has recently come to the forefront of media and public attention. Plagiarism comes in many forms, and ignorance is no longer an excusable defence. As students in this course, you will be held to the same academic standards as scientific researchers. Consequently, any violations will be prosecuted as outlined in the UCM Student Handbook.

Course Schedule

Generally, the course will have one lecture per week on Tuesday. The lectures offer the theoretical background for the tasks and are strongly recommended. There will also be time for discussion and questions. Two tutorial group meetings are scheduled per week: one on Tuesday after the lecture, and another one on Friday.

The definitive dates for deadlines and any changes to the lecture/tutorial schedule will be posted to EleUM, so you must check regularly.

	Tuesday (Lecture)	**Tuesday (Tutorial)**	**Friday (Tutorial)**	**Assessment**
Week 1 30 Oct – 03 Nov	Lecture 1: Matt Baker – Supramolecular Chemistry	Introduction Task 1 pre-discussion	Task 1 post-discussion Task 2 pre-discussion	
Week 2 06 Nov – 10 Nov	Lecture 2: Sabine van Rijt – Nanoparticles	Task 2 post-discussion Task 3 pre-discussion	Task 3 post-discussion Task 4 pre-discussion	
Week 3 13 Nov – 17 Nov	Lecture 3: Raimond Ravelli – Structural Biology	Task 4 post-discussion Task 5 pre-discussion	Task 5 post-discussion Task 6 pre-discussion	☐ Midterm paper deadline
Week 4 20 Nov – 24 Nov	Lecture 4: Tiffany Porta – iKnife	Lab visit Randwyck	Task 6 post-discussion Task 7 pre-discussion	
Week 5 27 Nov – 01 Dec	Lecture 5: Aurélie Carlier – Big Data	Task 7 post-discussion Task 8 workshop	Task 8 workshop Task 9 pre-discussion	
Week 6 04 Dec – 08 Dec	Lecture 6: Shane Ellis – Seeing Molecules	Task 9 post-discussion Task 10 pre-discussion	Task 10 post-discussion	☐ Final paper deadline
Week 7 11 Dec – 15 Dec				☐ Oral exam week

Lecture Descriptions

Lecture 1: Supramolecular chemistry
Instructor: Dr Matthew Baker

Topics

- Introduction to this course and your lecturers
- Molecules and beyond
- Self-assembly and molecular recognition
- Supramolecular polymers
- Functional supramolecular systems
- Supramolecular biomaterials
- Molecular machines

Description

The first part of the lecture will provide an introduction to this course. Various guidelines and the philosophy of the course will be presented.

The second part of the lecture will transition into supramolecular chemistry. Arising from observations and inspiration from nature, the origins and a brief history of major developments in the building of supramolecular structures will be presented. Heavily depending on organic and physical organic chemistry, recent developments into the creation of functional supramolecular systems will be presented. This will include a focus on the use of dynamic supramolecular interactions in the engineering of biomaterials.

Literature

Aida T, *et al*. Functional supramolecular polymers. *Science* 2012; 335(6070): 813–817.
Rosales, AM, *et al.* The design of reversible hydrogels to capture extracellular matrix dynamics. *Nat. Rev.* 1, 15012:

Related Tasks

Task 1: Prizes in science
Task 2: Drug development

Lecture 2: Nanoparticles
Instructor: Dr Sabine van Rijt

Topics

- The need for drug delivery
- Nanoparticles as drug delivery concepts; clinical development and historical perspective
- Development of nanotherapeutics, design principles
- Potential applications of nanoparticles in the field of regenerative medicine

Description

Drugs have long been used to improve health and extend lives. The practice of drug delivery has changed dramatically in the past few decades, and even greater changes are anticipated in the near future. The development of drug delivery systems that can control the rate at which a drug is released and the location in the body where it is released, provides great opportunities to facilitate and improve drug use in a clinical setting.

In the first part of this lecture, the focus is on the need for drug delivery and how nanoparticles can be used for this purpose. Different types of nanoparticles are discussed, along with their uses and how they can be modified to fit different purposes.

As a frontier discovery, we will discuss the utilization of nanoparticles in a clinical setting for the treatment of various cancers and their potential use in other diseases.

Finally, as an example of nanoparticle-based research being done at Maastricht University, we will discuss how the use of nanoparticles can improve regenerative medicinal approaches with a focus on their use in the development of new cell therapies and how they could help stimulate new tissue growth.

Literature

None

Related Tasks

Task 3: Size matters…
Task 4: The next frontier

Lecture 3: Structural biology
Instructor: Dr Raimond Ravelli

Topics

- The “what” and the “why” of structural biology
- Primary, secondary, tertiary and quaternary structures of biomacromolecules
- Structure determination methods
- Structure function relationship
- Antibody resistance
- Structure-based drug design

Description

The cell is the basic functional unit of life. Its molecular composition includes water, lipids, sugars, metabolites, proteins, RNA and DNA. Structural biologists are trying to decipher the function of these molecules by looking at their structure. More than one hundred thousand structures of macromolecules (proteins, RNA, DNA and complexes thereof) have been deposited in one central database (http://www.rcsb.org/). However, these only provide a glimpse of the complexity of the macromolecular content of a cell.

This lecture will provide an introduction to structural biology and its place in modern science. The first part of the lecture will introduce common structural themes used by nature. The methods used to determine these structures will be introduced. We will look at ribosomes to illustrate structure-function relationships. The second part of the lecture will give an outlook. How far are we with the exploration of the nanoscopic world of macromolecules and their interactions? What are the prospects towards a better understanding of this world, and how could this knowledge benefit human health?

Literature

Wilson DN. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nature Reviews Microbiology* 2014; 12(1): 35–48.

Related Tasks

Task 5: Extensively drug-resistant tuberculosis

Lecture 4: Intraoperative molecular diagnostics based on mass spec
Instructor: Dr Tiffany Porta

Topics

- Intraoperative clinical (tumour) diagnostics
- Molecular profiling
- Real-time mass spectrometry (imaging)
- Ambient ionization mass spectrometry

Description

There is a clinical need for new technologies that would enable rapid tumor diagnosis based on specific molecular signatures. In the current clinical setting and despite the large panel of technologies available at the Hospital, it remains difficult for the surgeon to determine whether he/she has completely removed cancer cells. Ambient ionization mass spectrometry has recently emerged for cancer diagnosis and surgical margin evaluation. For example, desorption electrospray ionization (DESI) has shown great promises to allow accurate evaluation of tumor margin from frozen sections within the duration of the surgery.

In addition, intraoperative molecular diagnostic based on mass spectrometry has recently gained attention from the medical field as it offers the possibility of *in vivo*, *in situ* and *real-time* mass spectrometric tissue analysis. For instance, in the context of liver or breast surgery; *real-time* information would guide and optimize surgical resection within the anatomic boundaries reducing the incidence of incomplete tumor resection – known as favorable to tumor recurrence.

We will discuss the technology behind the so-called “iKnife” and the major milestones to reach in order to perform successful intraoperative diagnostics. This course will also be illustrated by several examples published within the past few years demonstrating the capabilities of rapid evaporative ionization mass spectrometry (REIMS) to differentiate between non-tumor and tumorous tissues through the real-time MS analysis of smoke released during electrosurgical dissection. Discussion about the possibility of having these approaches soon become a routine medical tool for tissue diagnosis will follow.

The second part of the lecture will be a visit of the Division of Imaging Mass Spectrometry of the MultiModal Molecular Imaging (M4I) institute (Randwyck campus, UNS50). During the tour, you will discover state-of-the-art infrastructure and the possibilities it provides for personalized medicine. The equipment which will be implemented to the operating room of the MUMC+ for clinical research will also be presented to the students.

Literature

None

Related Tasks

Task 6: The Golden Ticket (related to the Randwyck visit)
Task 7: The iKnife: Hype or Hope?

Lecture 5: Big Data
Instructors: Dr Aurélie Carlier

Topics Part 1 (Dr Carlier)

- What is Big Data? The four V's: velocity, variety, volume, and veracity
- Big Data in medicine: High throughput experiments, -omics, precision medicine
- Big Data at UM: Radiomics and Materiomics
- Machine learning
- How are these techniques used in the analysis of Big Data

Description

We will begin the lecture with a brainstorm on Big Data, what is already known, which questions the students have. Then we will explain the characteristics of Big Data (the four V's: velocity, variety, volume and veracity) and why this technology is needed to tackle complex problems. In the second part, the basic machine learning techniques such as supervised and unsupervised learning are explained using intuitive examples. After introducing the general concepts of Big Data, we will apply them to Big Data in medicine. More specifically, we will introduce the students to the concepts of high throughput experiments, -omics technologies and precision medicine. Finally, we will highlight some examples where Big Data is improving the practice of medicine, drug discovery and biomaterials engineering, including research done at Maastricht University.

Literature

Leyens L, *et al*. Use of big data for drug development and for public and personal health and care. *Genetic Epidemiology* 2017; 41(1): 51–60.

Related Tasks

Task 8: Bone4Kids: Biomarker identification
Task 9: Don't fear the smart robots: the Google car

Lecture 6: Seeing molecules
Instructor: Dr Shane Ellis

Topics

- Molecular transformations in disease
- Molecular imaging
- Imaging mass spectrometry

Description

Molecules drive all biological processes including disease onset and development. To understand and diagnose diseases, researchers and clinicians require information about the molecular content of diseased tissues. It is not only which molecules are involved, but also where they reside in a tissue or cell. The area of research that seeks to understand this is referred to as molecular imaging, *i.e.*, the study of molecules, where they are located, and how they interact. In this lecture, you will learn about state-of-the-art molecular imaging technologies, their capabilities and limitations and how they are employed for cutting-edge research in Maastricht University.

Literature

Chughtai K and Heeren RMA. Mass spectrometric imaging for biomedical tissue analysis. *Chemical Reviews* 2010; 110(5): 3237–77.

Related Tasks

Task 10: Assessing kidney transplants

Tasks

Task 1: Prizes in science

Prizes are an integral part of scientific research. Not only does winning a prize recognize an individual (or group), but it communicates a clear message from the scientific community to the larger public. Currently, there are a plethora of prizes for a variety of scientific communities; however, some prizes carry much more importance and weight than others. We have the "genius prize," various "pre-Nobels," the Ig-Nobel prize, and a slew of recent mega-prizes looking to join the party.

Figure 1. The one that started it all.
Credit: timesofisrael.com

Figure 2. Watson and Crick and the structure of a DNA model. Who is missing?

This obsession with recognizing and rewarding achievement was started by inventor Alfred Nobel (1833–1896). With his dying will, Nobel left his fortune to the creation of the well-known Nobel Prizes. Some believe that the establishment of this prize was an apology to the world. Commencing in 1901, the Nobel Prizes have become a household name; however, many are unfamiliar with its origins, its exact purpose, or the method of choosing Nobel laureates and they are often shrouded in controversy.[1]

Many Nobel laureates and their work become quite famous. Sir Alexander Fleming was awarded in 1945; Watson and Crick were awarded in 1962; Jean-Marie Lehn was awarded in 1987. Each of these awards represents a significant breakthrough in Chemistry or Physiology and Medicine, leading to different ways we approach the treatment and understanding of the human body and its diseases.

Just this last year, one of our own academic neighbours was awarded the Nobel Prize. Awarded for the design of molecular machines, Ben Feringa, an organic chemist from Groningen, shared the 2016 Nobel Prize with Sir Fraser Stoddart and Jean-Pierre Sauvage.

As quoted during the 2016 Nobel announcement: "Bernard Feringa was the first person to develop a molecular motor; in 1999 he got a molecular rotor blade to spin continually in the same direction. Using molecular motors, he has rotated a glass cylinder that is 10,000 times bigger than the motor and also designed a nanocar. 2016's Nobel Laureates in Chemistry have taken molecular systems out of equilibrium's stalemate and into energy-filled states in which their movements can be controlled.[2] In terms of development, the molecular motor is at the same stage as the electric motor was in the 1830s, when scientists displayed various spinning cranks and wheels, unaware that they would lead to washing machines, fans and food processors. Molecular machines will most likely be used in the development of things such as new materials, sensors and energy storage systems." *(continued on the next page)*

[1] Popkin G. Update the Nobel Prizes. *The New York Times*. October 3, 2016.
[2] Browne WR, Feringa BL. Making molecular machines work. *Nature Nanotechnology* 2006; 1(1): 25–35.

Molecular machines, such as those developed by the Nobel laureates, have immense potential in our future lives. Yet, to date, only a handful of researchers know how to make and use such devices. The future remains very bright and promising for the next generation of researchers to take these discoveries and realize functional materials, drugs, and products.

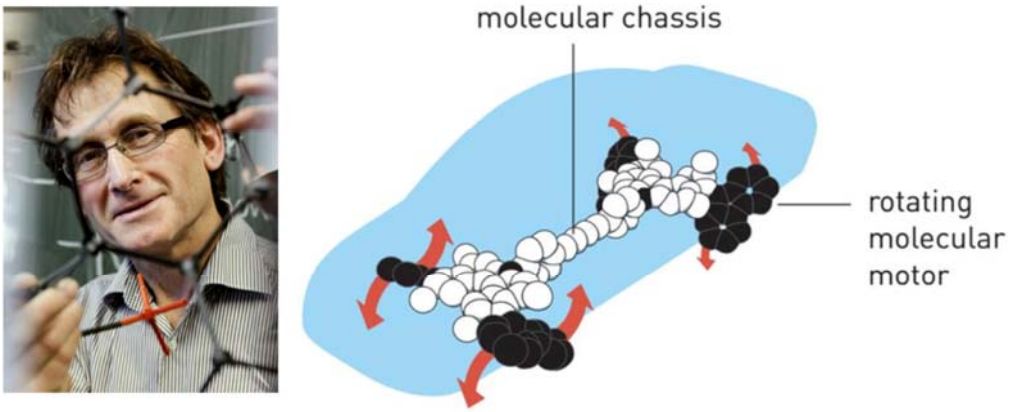


Figure 3. Ben Feringa and his famous molecular motorcar.

Task 2: Drug development

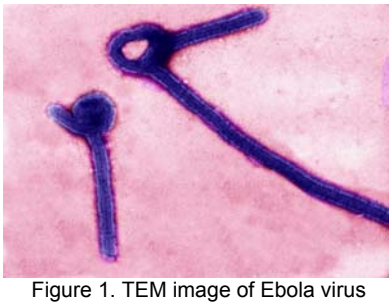


Figure 1. TEM image of Ebola virus particles. Credit: USAMRIID and Reuters

In December 2013, a 2-year-old boy died in the village of Meliandou, Gueckedou Prefecture. Quickly thereafter, his mother sister and grandmother became ill. A year later, the Ebola virus has killed almost 7,000 people and infected 20,000. In January 2016, Merck developed a "miracle vaccine," designed to prevent future outbreaks of this lethal disease.[3] Even 10 years ago, a response this swift and quick would be unthinkable.

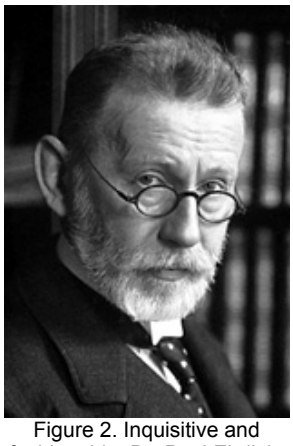


Figure 2. Inquisitive and fashionable, Dr. Paul Ehrlich.

Although vaccines and drugs are fundamentally different, the scientific efforts in their design have become indistinguishable. Medicinal compounds have been in use since ancient civilizations, with well-known written records being produced in both ancient Chinese and Greco-Roman times. These discoveries and uses were mainly empirical in nature and were passed down extensively *via* oral tradition. However, it is only in the past 60 years that searching for new drugs has transitioned into the scientific realm.[4]

This scientific development of small molecule therapeutics had an immediate benefit. In 1900, 1/3 of all deaths were from pneumonia, tuberculosis, and diarrhoea. By 1940, the chances of survival were 91%; by 2000 the chance was 96%. While various factors, including sanitation, contributed to the increase in life expectancy – from less than 50 years in 1900 to over 75 in 2000 – rational discovery and design of drugs undoubtedly made an impact.

Starting with Paul Ehrlich's observation (ca. 1874) that certain dyes have an affinity for biological tissue, and fuelled by the discovery of miracle dugs like penicillin, our knowledge of the way small molecules interact with the human body has grown exponentially. Advances in organic chemistry, structural biology, and analytical chemistry led to rapid growth of the drug development field. Techniques such as high throughput screening, structure/property relationships, and combinatorial chemistry led to the boom of the drug industry in the 80s and 90s giving birth to giant companies like Merck and GSK (as they are known as today).

(continued on the next page)

[3] Kupferschmidt K, Cohen J. Ebola drug trials lurch ahead. *Science* 2015; 347(6223); 701–702.
[4] Drews J. Drug discovery: a historical perspective. *Science* 2000; 287(5460); 1960–1964.

However, this boom is no more. Much of the pharmacological chemical space has been sampled, leaving little novelty in new drugs. Coupled with tough regulatory pressure, changing medical policy, and patent expiration, many companies have drastically reshaped and changed strategies. Currently, many scientific efforts for drug discovery go far beyond making small molecules to engineering molecular assemblies, nanoparticles, viruses, and biomolecules. Furthermore, many academics and companies are working to pool this knowledge and allow computers to predict and/or design new compounds and objects.

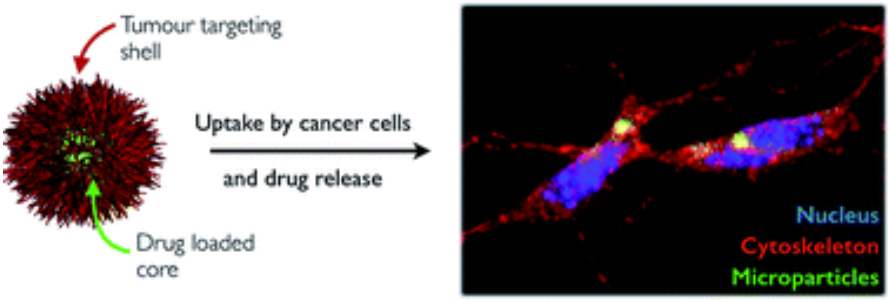


Figure 3. Nano-objects hold promise for future drug development. Credit: DOI: 10.1039/C4RA16593D

Task 3: Size matters…

The concept of nanoparticles and drug targeting was inspired by a visit of one of the giants in science – Paul Ehrlich – to Karl Maria von Weber's opera "Der Freischütz". In this opera, so-called "Freikugeln", which are made by calling the spirit of the devil, play a central role. These Freikugeln always hit their goal, even if the rifleman did not aim properly or if the goal was out of reach. Paul Ehrlich, who had been long working on the staining of bacteria and tissues, thought that this concept of targeted delivery could greatly improve drug therapy. He called the delivery system that would be used in this type of therapy "Zauberkugeln" – or "Magic Bullets".

One of the pioneers in this field was Professor Peter Paul Speiser at the ETH (Swiss Federal Institute of Technology) in Zürich. His strategy for delaying and controlling drug release was based on the development of miniaturized delivery systems based on nanoparticles.

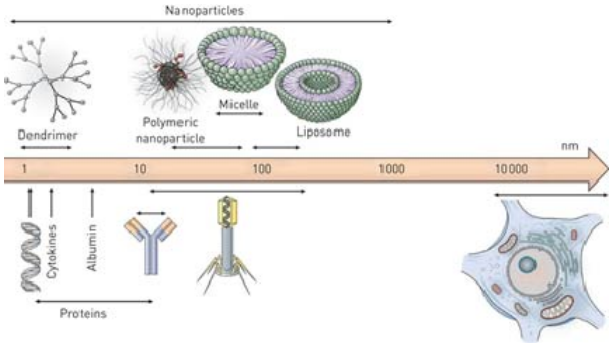


Figure 1. Nanoscale in biology
Credit: DOI: 10.1183/09031936.00212813

Nanoparticles are small compared to most particles, but are large compared to molecules. The size of nanoparticles is essential for their drug delivery capability and their ability to interact with other biological entities. Perhaps the most publicized use of nanotechnology in drug delivery is the use of nanoparticles to deliver drugs to cancer cells.[5] Nano-sized particles are engineered to be attracted to diseased cells, which allows for the direct treatment of those cells. This technique reduces damage to healthy cells and other systemic effects.

Several different types of nanoparticle formulations are currently used to treat cancer patients and many nanoparticle formulations are currently in clinical trials.

New generations of nanoparticles are equipped with additional "smart" features so they can bind to specific biomolecules and/or respond to internal or external stimuli at the right moment in time, a bit like nano-bots.[6] This gives them interesting features for applications in medicine and the biomedical sciences.

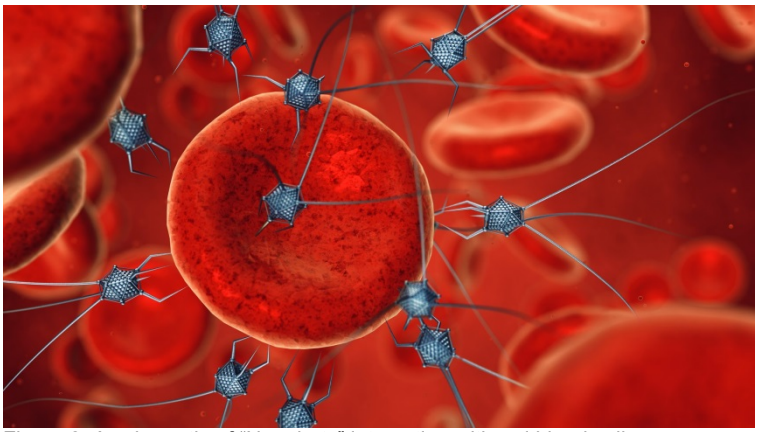


Figure 2. A schematic of "Nanobots" interacting with red blood cells.
Credit: www.alamy.com-F3EMEB

[5] Peer D, *et al*. Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotechnology* 2007; 2(12): 751–760.
[6] van Rijt SH, *et al.* Protease-mediated release of chemotherapeutics from mesoporous silica nanoparticles to ex vivo human and mouse lung tumors. *ACS Nano* 2015; 9(3): 2377–2389.

Task 4: The next frontier

It has been projected that chronic diseases will account for almost three-quarters of all deaths worldwide by 2020. Already today, chronic disease is a massive burden on health care systems. Patients with chronic conditions account for most primary care consultations in the developed world. One could argue that the next frontier is neither Mars nor the outer limits of the universe; it is our health.

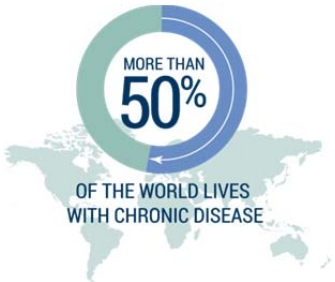


Figure 1. Chronic disease burden in the western world
Credit: http://cmcd.sph.umich.edu/

Regenerative medicine is a field where very exciting research is happening. For example, stem cells are being used to promote the body's natural healing process.[7] This could be a potential cure for many of the prevalent chronic diseases and degenerative disorders plaguing our society. But despite this high potential, few stem cell therapies have become available to humans.[8] There are a few reasons for this, but one of the major factors is that it is currently largely unknown what happens to these cells after implantation.

It follows that many scientists are currently developing new tools to permit *in vivo* imaging for real-time assessment of implanted/transplanted cells. Such information is critical to modulate and tailor the therapy for each patient. Nanoparticles could be used as such a tool, but they first need to be designed to allow for long-term imaging throughout the body without toxic side-effects. This is not an easy feat![9]

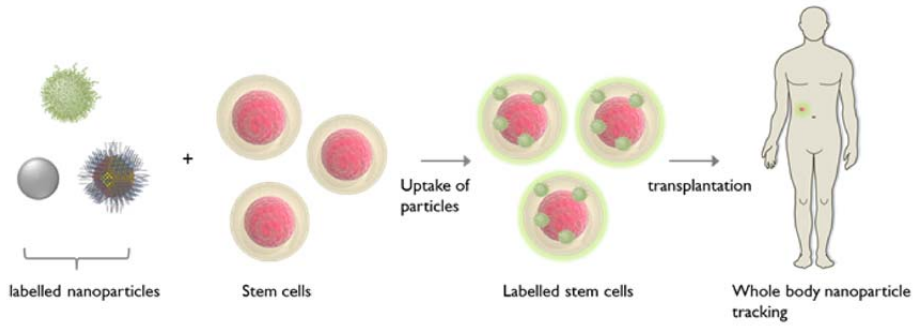


Figure 2. Nanoparticles for the tracking of cells. Credit: Dr. Sabine van Rijt

[7] Trounson A, DeWitt ND. Pluripotent stem cells progressing to the clinic. *Nature Reviews Molecular Cell Biology* 2016; 17(3): 194–200.
[8] Trounson A, McDonald C. Stem cell therapies in clinical trials: progress and challenges. *Cell Stem Cell* 2015, 17, 11–22.
[9] Ngen EJ, *et al.* Imaging transplanted stem cells in real time using an MRI dual-contrast method. *Scientific Reports* 2015; 5: 13628.

Task 5: Extensively drug-resistant tuberculosis

The National Institutes of Health facility in Bethesda recently received a patient who was suffering from a rare, extensively drug-resistant form of tuberculosis (XDR-TB). She was transferred *via* special air and ground ambulances from a hospital in Chicago and was moved to an isolation room specifically designed for handling patients with respiratory infections, including XDR-TB. The patient travelled widely in the weeks before she was diagnosed with the disease, including a trip to India. Health officials had to track down hundreds of people who could have been at risk during her trips, including plane passengers and people at Chicago O'Hare airport. XRD-TB is very rare in the U.S. but also very dangerous.[10] Only about one third to half of XRD-TB cases can be cured.

Tuberculosis is as old as mankind. In Europe, it peaked in the 1800s, when it caused nearly 25% of all deaths. Famous people that died too young of this disease include John Keats, Franz Kafka, George Orwell, Henry Purcell, Giovanni Pergolesi, and Igor Stravinsky. Robert Koch (Nobel Prize 1905) identified a bacillus, *Mycobacterium tuberculosis*, as being the cause of the disease. For more than a century, sanatoria offered rest, good nutrition, sunlight and fresh air to treat patients suffering from diseases such as tuberculosis. It wasn't until 1943 that the first antibiotic against tuberculosis was found, streptomycin (Nobel Prize 1952).

One in three people in the world is infected with *Mycobacterium tuberculosis*, however, people only become ill when the bacteria become active. TB is nowadays treated with a course of first-line, anti-TB drugs. If these drugs are misused or mismanaged, a multidrug-resistant TB (MDR-TB) can develop. These can be treated, albeit more difficultly (*e.g.* with more side-effects and a higher cost) with second-line drugs.[11] If these drugs are also misused or mismanaged, XDR-TB can develop. XDR-TB raises concerns of a future TB epidemic with restricted treatment options.

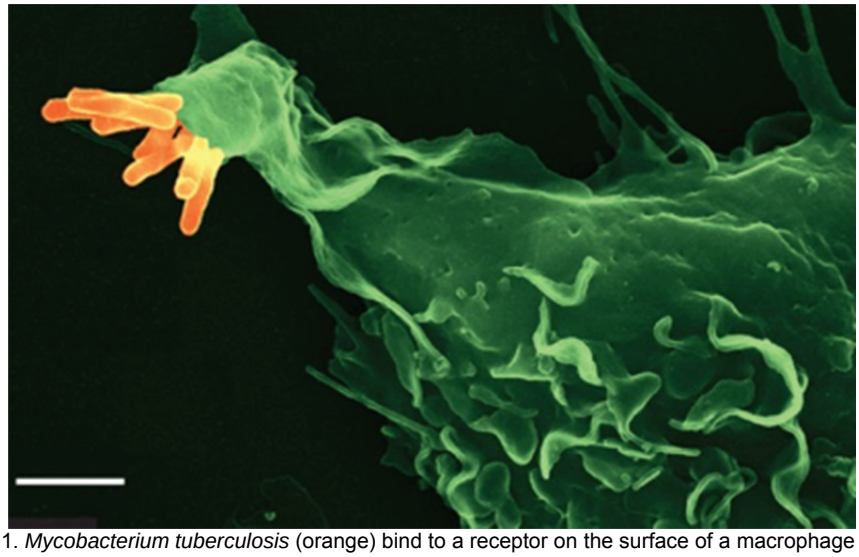


Figure 1. *Mycobacterium tuberculosis* (orange) bind to a receptor on the surface of a macrophage (green)

Task 6: The Golden Ticket

[10] Russell DG, *et al*. Tuberculosis: what we don't know can, and does, hurt us. *Science* 2010; 328(5980): 852–856.
[11] Manson AL, *et al.* Genomic analysis of globally diverse Mycobacterium tuberculosis strains provides insights into the emergence and spread of multidrug resistance. *Nature* 2017; In Press. DOI:10.1038/ng.3767.

Task 6: The Golden ticket

A la *Charlie in the Chocolate Factory*, you have been presented with a Golden Ticket. You have a once in a lifetime chance for a fully paid 4-year PhD at the world-renowned M4I or MERLN Institutes at Maastricht University. As with all things, there is a catch. You have to decide within 48 hours, or you lose the opportunity. Naturally, you are a good student, but opportunities like this do not arrive every day. How do you quickly decide if this opportunity is the one for you, or if you should decline for better opportunities down the road?

Although you have seen some talks from researchers in this institute, what is it that they do all day? You know that MERLN talks about regenerative medicine and M4I talks about mass spec imaging, but so do many other research groups and institutes worldwide. Special techniques like the iKnife, fast image acquisition, and lipid isomer resolution are all special to M4I, but sometimes the working principles behind them are a bit vague. Likewise, MERLN has specialties in bone regeneration, bio/microfabrication, and cell topography that sound really interesting, but are hard to define right now.

Even if science is the core of a PhD, as with any job other factors also take a role in your decision and productivity. Environment, facilities, funding, and long-term vision are all aspects that become crucial to your success down the road. It is often hard to determine what matters most, and even more difficult to find out what you need to know.

These days, institutes and research groups produce a lot of content in the form of papers, publications, media coverage, websites, Twitter, Instagram, and Facebook accounts. Now luckily, you are given the chance to visit the labs within MERLN and M4I, giving you only a few hours to gather extra information, leading you to a more confident decision.

This task may be a bit of a stretch, but it is a very realistic situation in your future. Very quickly and with a high degree of confidence, you need to be able to assess the quality of a workplace along with the fit for your personal goals and working style, often under extreme time pressure.

Now enough. What is your decision?

Figure 1 To PhD or not to PhD…PhD comics #282. Copyright Jorge Cham.

Task 7: The iKnife: Hype or Hope?

In 2013, BBC news reported that “an "intelligent" knife that can sniff out tumours to improve cancer surgery has been developed by scientists”. The same year, the Daily Telegraph echoed that “an "intelligent" knife that knows when it is cutting through cancerous tissue is being tested in three London hospitals.” With the iKnife – sometimes qualified as the “Bistoury of the future” – the surgeon would have the potential to achieve tumour removal with 100% of certainty and accuracy.[12,13]

The potential of the iKnife is huge. Additional surgical procedures (which are associated with low long-term survival rates) that are required in the case of incomplete resection would no longer be performed. And the patients – who are already facing a life-changing diagnoses – could benefit from fewer procedures, faster recovery, and an overall improved quality of life post-surgery.

In 2014, the iKnife was once again described as a scientific breakthrough by The Telegraph magazine, but the technology is still not adopted in clinical practice as of 2017.

So everyone has the right to wonder: is the iKnife hype or hope? Does it deserve this level of press attention? And is the iKnife still on its way to revolutionize modern medicine and cancer surgery?

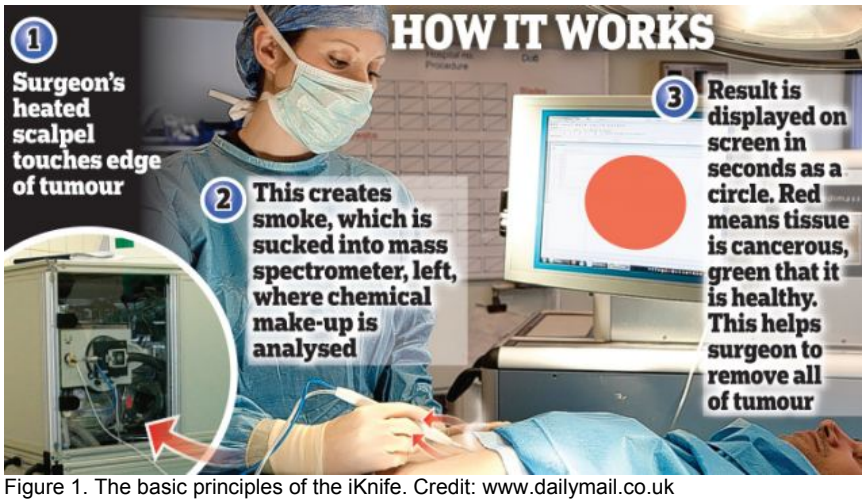


Figure 1. The basic principles of the iKnife. Credit: www.dailymail.co.uk

[12] Balog J, *et al*. Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. *Science Translational Medicine* 2013; 5(194): 194ra93.
[13] Ifa DR, Eberlin LS. Ambient ionization mass spectrometry for cancer diagnosis and surgical margin evaluation. *Clinical Chemistry* 2016; 62(1): 111–23.

Task 8: Bone4Kids: biomarker identification

Congenital pseudarthrosis of the tibia (CPT) is a rare disease characterized by a progressive bowing of the tibia that develops into spontaneous fractures in the distal third of the tibia. Typically the endogenous bone regeneration is insufficient, resulting in a pseudarthrosis, which is treated by physical excision of the abnormal bone tissue (called the 'hamartoma') and internal or external fixation. In recent clinical practice, rhBMP-2 or rhBMP-7 (recombinant human bone morphogenetic protein) are used to enhance the surgical outcome. However, the effectiveness of BMP therapies is still highly debated and the FDA has issued a warning against the use of BMP in skeletally immature patients.

To augment the limited amount of clinical data associated with this orphan disease, a virtual data-set, consisting of 200 virtual patients receiving both a treatment and no treatment, was created with an advanced computational model of bone regeneration.

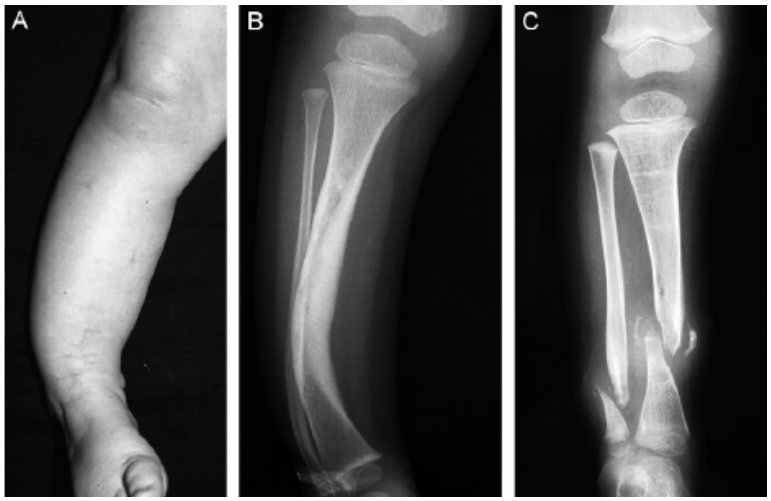


Figure 1. Clinical (A) and radiographic (B) heterogeneity of congenital pseudarthrosis of the tibia (CPT) with anterolateral bowing of the leg and atrophic pseudarthrosis of the lower third of the leg (C). Pannier S. Congenital pseudoarthrosis of the tibia. Orthopedics & Tramatology: Surgery & Research 2011; 97(7): 750–61.

http://www.bone4kids.be/en/

In this task, we will not focus on the biological aspects of CPT but you will learn how to analyze the dataset of 200 virtual CPT patients. Therefore, you will first get a small introduction to R, a statistical programming language. Then you we will learn how to import the dataset into R and explore the data using a variety of supervised and unsupervised machine learning techniques. The assignment is continued on the next page…

Task 8 assignment

Intro to R

- Download and install R: https://cran.r-project.org/bin/windows/base/
- Download and install R studio: https://www.rstudio.com/products/rstudio/download/#download
- Online tutorial 1: https://rpubs.com/isabelduarte/marineGenomics2016
- Tutorial 2 (Optional): https://cran.r-project.org/doc/contrib/Torfs+Brauer-Short-R-Intro.pdf

Clinical dataset

Characteristics of the dataset:

- 8 molecular markers (first 8 columns)
- 200 patients (rows)
- CI-index: “complexity index” – measure of severity; a high index is associated with a worse clinical outcome

Questions:

- Is the BMP-2 treatment beneficial in this dataset?
- Is the treatment beneficial for all patients?
- Can we stratify the patients?
- Do the patient groups have common characteristics? What are these characteristics?

Tasks:

STEP 1: load the required packages and the dataset

- Explore the dataset. What are the dimensions?

STEP 2: investigate the CI index with and without the treatment.

- Is the BMP-2 treatment beneficial?

STEP 3: data exploration

- Do all patients respond similarly?
- Try out k-means clustering to automatically identify patient groups.
- What is the effect of different k’s?
- Do you always get the same clusters (even with the same k)?
- Make a silhouette plot to explore the optimal number of clusters.
- Try out hierarchical clustering.
- What is the effect of different distance metrics?
- What is the effect of different linkage methods?

STEP 4: Supervised clustering

- Based on clinical knowledge we classify the patients in 4 groups:
    - non-responders: CI-before >0.5; CI-after >0.5
    - responders: CI-before >=0.5; CI-after <0.5
    - asymptomatic: CI-before <0.5; CI-after<0.5
    - adverse responders: CI-before<0.5; CI-after>0.5
- Do we retrieve the same groups using hierarchical clustering?

- Which patients are not correctly classified? Why?
- How does the distance matrix or linkage method affect the incorrect classification?

STEP 5: Decision tree

- Do the patient groups have particular cellular characteristics that we can use as a biomarker?
- *Optional: prune the decision tree*
- *Optional: plot the decision tree boundaries*
- *Optional: use a random forest classifier instead of a decision tree*

Task 9: Don’t fear the smart robots: the Google car

Figure 1. The self-driving car of Google (https://www.google.com/selfdrivingcar)

Fred: Hi Tom, did you see the movie “I, Robot” last night on television? I really liked it, robots becoming more intelligent than humans, a bit scary though, with their potential to murder us…

Tom: Ah, I also saw it, although without my wife, she does not like these scary sci-fi movies.

Fred: It is not that sci-fi anymore, you know, I recently read an article on the self-driving car of Google and how software developers need to program the car how to act in the event of an unavoidable accident: kill the driver, one person, or kill ten pedestrians crossing the street? Something similar also happened in the movie, remember? The robot decides to save Will Smith’s character and lets the little girl die because her chances of survival were lower.

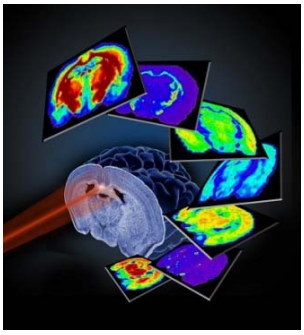
Tom: Seriously? Who will buy a car or robot that can potentially kill him?

Fred: I know, but on the other hand, there are so many fatal traffic accidents today, using this big data technology we could potentially save so many people, isn’t that more important? So far these cars have driven over 1.2 million miles without any accidents, pretty amazing, no?

Tom: It definitely is, but sometimes these advances in technology are a little creepy. Not just because of the “robots taking over”. People could start using it to control others like in that short movie we watched a few weeks ago, Sight*.

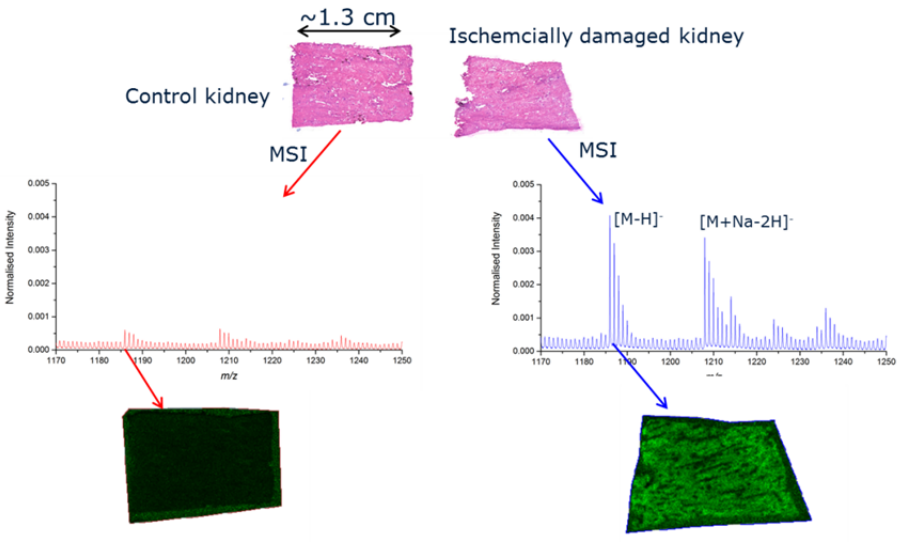
* https://www.youtube.com/watch?v=lK_cdkpazjI

Task 10: Assessing kidney transplants



Mass spectrometry imaging provides a powerful combination of molecular specificity, sensitivity and resolution for unlabelled molecular imaging. Mass spectrometry is concerned with the detection and identification of molecules based on their characteristics mass (a scale for molecules). Traditionally mass spectrometry was used by organic chemists for working out the structure of purified organic molecules. For this it was very useful as the ionisation methods used were energetic which resulted in the breaking of bond during analysis and by studying how molecules broke the structure could be pieced together. However, there was a strong need for new approaches that could detect intact molecules as large as proteins without purification and without breaking the molecule. In the mid 1980s two such techniques were reported, electrospray ionisation (ESI) and matrix assisted laser desorption ionisation (MALDI). Now for the first time intact molecules (such as lipids, peptides and proteins) could be observed from complex mixtures, even directly from tissues and cells and paved the way for modern mass spectrometry imaging.[14]

Given the first thing to change is the biochemical processes within the tissue, and the fact that diseased/damaged tissue are characterised by a change in molecular composition and distribution, mass spectrometry imaging resents an exciting tool to study and identify sick tissues. [15] One potential application area is assessing the quality of kidney transplant. There is a constant need to increase the supply of suitable kidneys for transplant. With this need the acceptance criteria for kidneys is often extended to so-called “extended criteria donors” who have cardiac death. As a result tissue is exposed to acute ischemic damage- damage caused by a lack of blood and oxygen flow. Tissue must be assessed fast as there is a very small window for a transplant to be initiated. Surprisingly it is almost impossible for histologists to identify and quantify acute ischemic damage, in part because it occurs on such a fast time scale before histological changes may occur. Other methods are clearly needed.



[14] Addie RD, et al. Current state and future challenges of mass spectrometry imaging for clinical research. *Analytical Chemistry* 2015; 87(13): 6426–33.
[15] Rao S, et al. Early lipid changes in acute kidney injury using SWATH lipidomics coupled with MALDI tissue imaging. *Renal Physiology* 2016; 310(10): 1136–47.