KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

Bioscopy

Following the building blocks of life at work inside living cells

"KNAW- Agenda Grootschalige Onderzoeksfaciliteiten"

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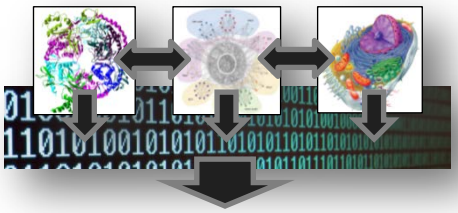
Summary

Bioscopy is envisioned to be an integrated infrastructure encompassing the research facilities necessary to understand life from single molecules all the way to living cells. Bioscopy aspires to reach the next frontier in life sciences, namely the ability to understand and perturb the structure, activity, dynamics and interactions of individual biomolecules in their natural cellular environment in real time and at multiple scales, providing spatiotemporal understanding and control to the chemical processes that constitute "life". Achieving these goals will require substantial technological advances in different disciplines as well as integration of various state-of the art technologies.



Bioscopy will develop innovative approaches to study molecules at the atomic level and integrate structural biology methods with cell imaging techniques. The sensitivity and the throughput capacity of different "omics" technologies will be expanded to enable routine analyses at the single cell level. A key aspect of the Bioscopy infrastructure will be the integration of different approaches at the experimental level and also at the level of data handling and analysis. By bridging the gaps between technologies and research fields and merging different approaches into networks that would allow analysis of biomolecules at different scales, both in scale and in time, we seek to revolutionize our knowledge of the inner workings of living cells. In the next 12 to 15 years, Bioscopy can create the technological base and provide open access to methodologies that will allow to bring **structural biology to the (sub)cellular and molecular levels**, to truly **merge multiple "-omics" technologies,** to **develop state-of-the art computational and modeling approaches** and to integrate the knowledge generated by all these technologies at the experimental and data level. Ultimately this will make it possible to create advanced models of the biomolecular activities occurring inside cells, which can describe the chemical networks that make a live cell. This will allow testable new hypotheses to be formulated, and provide the basis to manipulate individual components of a cell to understand biological processes in health and disease, and bring therapeutic strategies and bio-based technologies to a next level.

Keywords

Biomolecules, cells, life sciences, microscopy, structural biology, bioinformatics, "-omics"

II. INHOUDELIJKE UITWERKING

A. SCIENCE AND TECHNICAL CASE

– Beschrijf in hoeverre het hier een geheel nieuw idee betreft of een verbetering of opvolging van een reeds bestaande faciliteit.

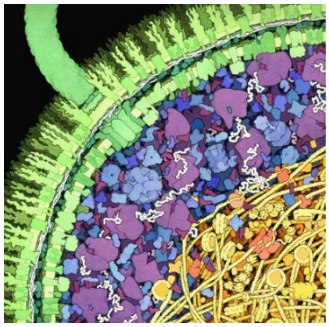
Bioscopy embodies a necessary logical step in the development of research facilities in Life Sciences. It aims at the development of individual structural biology, imaging and "-omics" technologies to a level where these technologies overlap and merge. For example, determination of a structure of a particular macromolecule in its natural cellular environment will be tightly connected to studying the dynamics, modifications and interactions of the same molecule in a given cell using "-omics' techniques like mass spectrometry and imaging approaches such as super-resolution microscopy. The core aspect of Bioscopy is the profound integration of the outputs created by different technologies, their multiscale analysis and the generation of comprehensive quantitative models based on these data. Bioscopy will be founded on the state of the art techniques, but integration of these techniques will catalyze their transformation towards the intra- and interdisciplinary level.

In the Netherlands, all of the technologies involved are available at very high scientific level. Furthermore, the geographical proximity of the different participating centers of expertise and the existing extensive network of collaborations between these centers puts the Netherlands in a unique and excellent position to establish one infrastructure where all these technologies are brought together. While individual collections of technologies exist also in other countries, integrating all of these technologies in a single infrastructure will be truly unique world-wide and will bring the Netherlands to the top of the molecular and cellular life sciences. Bioscopy aspires to bring together scientists from Utrecht University, the University Medical Centre Utrecht, the University of Amsterdam, the Amsterdam Medical Center, Leiden University, Leiden University Medical Centre and the Netherlands Cancer Institute, who will join forces, as a very strong nucleus of the required technologies exists at these locations, and serve as a catalyst for future participation of other scientists in the Netherlands both as developers and users of the infrastructure.

Science Case

– Geef een algemene introductie van de wetenschappelijke waarde van de faciliteit.

Understanding how biomolecules and cells work in both healthy and diseased states, is key to allow scientists working in animal, plant and medical science to eventually develop truly novel therapeutic approaches, inventing more effective medicines and early stage diagnostics strategies, and revolutionize bio-based technology, creating plants with improved qualities or bacteria that produce key materials. This knowledge will generate a better understanding of life and will foster entirely novel solutions for bio-inspired sustainability, healthy food and other major societal challenges. No single technology will be able on its own to find the answers to the current intricate life science questions. Inside cells, many different biomolecules interact with each other and while technologies studying specific types of biomolecules in cells are commonplace, only a truly integrative approach for molecular and cellular life sciences will allow us to push the boundaries of life science research drastically further over the decades.



Painting by David Goodsell, illustrating the complexity of interactions in living cells (2011)

Many basic, important scientific questions in fundamental life sciences, such as "how do drugs work inside cells?" or "what is the molecular origin of diseases such as Alzheimer, ALS and autoimmune diseases?" still remain largely unanswered, simply because we do not yet have the proper combination of tools and methods to answer them. Bioscopy will represent an infrastructure that allows addressing and answering questions such the following, which all were defined in the 2015 National Science Agenda (Nationale Wetenschapsagenda):

- "How does the nervous system develop and how can degenerative processes be countered?"
- "Every tumor is different: how can we understand the disease cancer well enough to develop a therapy for each form of cancer?"
- "Big data: can we use large datasets to obtain useful information on scientific questions?"
- "Can we build a synthetic cell?"
- "How did life originate and how does evolution work?"
- "How can we better understand the properties, functionality and interplay of molecules in living systems?"
- "How do cells work and what do they tell us about the processes of life?"
- "Can we develop new food crops that produce more food with less use of harmful chemicals?"

– Beschrijf de wetenschappelijke voordelen en verwachte doorbraken.

Society is dealing with great future challenges regarding novel challenges in the health of a population that reaches a seemingly ever-expanding life expectancy and the sustainability of food supply for the increasing world population. The sequencing of the genome of humans and many other organisms offers unique opportunities to improve health and to stimulate scientific and biotechnological activity. With extensive knowledge of the genomes available, emphasis is now rapidly moving to the biological interpretation of the genome sequence information and variation. This biological interpretation encompasses the immense task of identifying structure, function and interactions of a diverse set of biomolecules, and of their role in biological processes inside living cells. The understanding of how cells function at atomic, molecular and cellular level is heavily reliant on technological advances for the analysis of biomolecules in the cellular context. It demands the application of evolving multidisciplinary technologies to enable the characterization of biomolecules with respect to their structures, interactions, abundance, localization modifications and deciphering the networks that relate them to achieve their cellular functions. These increasingly complex biological and biomedical questions of the future will need well-trained scientists and cutting edge, integrated technologies, to be answered. We need to evolve and integrate existing technologies, to be able to study molecules and their assemblies that eventually make up a living cells, at a variety of spatial and temporal scales.

Currently, no methods exist yet that can provide an integral detailed view on complex cellular mechanisms. High-resolution methods can typically zoom in on a single type of molecule, which should be available in large quantities in a purified form. Methods that address increasingly more complex pictures, lag behind in the resolution that is required to unravel the underlying molecular mechanisms. By developing and integrating the different technologies of Bioscopy, it will become possible to provide a global picture of activities of biomolecules with the desired high spatial and temporal resolution. Connecting molecular properties with cellular behavior and environmental cues in a spatiotemporal fashion is one of the largest challenges in biology and highly important for understanding diseases and the development of treatments thereof.

Each of the different technologies that will be integrated in Bioscopy will provide specific contributions to achieve breakthroughs in many different biomedical and biological research areas. Integration of these technologies at various levels (see *Technical case*) is necessary to make a next step in our conceptual understanding of how molecules work in live cells.

The type of questions that we would like to address in the future, and to which Bioscopy can contribute are:

- Which nanostructure(s) does a biological macromolecule of interest adopt in its cellular environment?
- What are the molecular interactions that a particular molecule engages in the natural cellular environment and how frequent and transient are they?
- Does the molecule or the complex of which it is part change during its function?
- How do molecules and their supramolecular complexes move and change during the lifetime of a cell to achieve their function?
- How are the dynamic interactions between molecules, the cytoskeleton and biomembranes regulated?
- How does the molecular behavior of a molecule change in a diseased state and how is it affected when the cell is treated with a drug?
- How do drug molecules behave when performing their action and can we use this knowledge for the optimization of drugs or treatment regimens?

With the ever-increasing complexity of systems being studied in life sciences, it is clear that only a synergistic combination of experimental techniques and computations will allow tackling these major challenges. Advanced data analysis and modeling will play an central role in order to integrate information from different technologies and cope with the ever increasing flow of data being generated. By integrating data from many different sources we expect that we will be able to understand the vast complexity of the activities and interactions exhibited by biomolecules that together compose cells.

– Beschrijf hoe deze faciliteit zich verhoudt tot alternatieve faciliteiten/onderzoeksmethoden.

Currently, many experimental approaches use either a single technology or a limited combinatorial subset of technologies to answer scientific questions. By providing concerted, integrated access to a multitude of technologies and enabling integrated analysis of the resulting data, Dutch life science researchers will open new research applications and make most efficient use of available technology. Combining multiscale technologies on the study of a single molecule or even applying them on the same sample, will yield a unique, integrative view on molecular functioning that cannot be obtained when techniques are applied alone. Making this step forward is necessary to keep the Netherlands internationally competitive in the molecular life sciences. Below is an overview of the different technologies that form the basis for Bioscopy. *The future developments envisaged in Bioscopy are described in the Technical case*.

- **X-ray crystallography** allows the determination of the structure and function of many biological molecules, including vitamins, drugs, proteins and nucleic acids (DNA or RNA) and their interactions, at very high, atomic resolution. Bioscopy includes Gros, Sixma and Perrakis as experts in X-ray crystallography.

- **NMR** is a well-established method to non-invasively study structure and dynamics of molecules at the atomic level and recent developments have created novel opportunities to obtain such information in complex molecular environments such as cellular organelles, cells and cellular tissue. Bioscopy includes Boelens and Baldus as experts in NMR.

- **Electron microscopy** allows imaging of objects that range from large molecules to cells, with resolution from a few Ångstrom (for single molecules and their complexes) to a few nanometer (for sub-cellular volumes. **Single particle Electron Microscopy**, allowing macromolecular complexes to be studies at atomic resolution, has been named Method of the Year by the Nature group of journals. **Correlative light-electron microscopy (CLEM)** allows examination of the same sample using both electron and light (fluorescence) microscopy, providing a connection between different levels of imaging and sample complexity, is also rapidly advancing. Bioscopy includes Klumperman, Förster and Koster as experts in electron microscopy and CLEM.

- **Fluorescence microscopy** provides direct information on the localization, structure, conformation, dynamics and interactions of biological molecules in living cells. Bioscopy includes Akhmanova, Gadella, Gerritsen, Hoogenraad, Jalink, van Rheenen and Stoorvogel as experts in fluorescence microscopy.

- **In-vivo imaging** allows the detection and monitoring of biomolecules inside living organisms, ranging from small organisms such as worms to mammals, in particular mice. Unique is the expertise in 3D single cell analysis and tracing of organoids, both in vivo and in vitro. Bioscopy includes van Rheenen and van den Heuvel as experts in in-vivo imaging and Snippert in organoid microscopy and tracing.

- **Probe development** is an important direction in advancement of different imaging approaches. Among novel probes suitable for different imaging methodologies, **Nanobodies** (monomeric antibody fragments, which, like a whole antibody, can bind selectively to a specific antigen) are highly promising tools due to the smaller size and ease of producing in large quantities and labeling. Bioscopy includes Gadella as an expert in fluorescent probe & biosensor development and van Bergen en Henegouwen as a nanobody expert.

- **Biophysical and biochemical approaches for biomolecular interactions** allow the characterization of the interaction between biomolecules with each other (e.g. between a transcription factor and DNA) or with inhibitors (e.g. a target protein with a drug candidate) to monitor complexes from a mechanistic viewpoint: to measure the affinity between the complex components, the half time of the complex, and the transient kinetics of complex formation (in the first few milliseconds of a reaction), that allow to fully understand the mechanism of action of these molecules in the spatiotemporal domain. Bioscopy includes Braakman, Sixma, Perrakis, Fish, Gadella and Jalink as experts in biophysical and/or biochemical characterization.

- **Genetic approaches** allow the comprehensive analysis and understanding of genetic and epigenetic information, chromatin conformation, protein-DNA interactions, expression level of mRNAs (transcriptomics), screening for genes or substances that alter a phenotype, to study how the cellular environment impinges on biomolecules and can be applied even in the level of an individual cell. Bioscopy includes, Kloosterman, de Laat, Kerkhoven and Cuppen as experts in next-generation sequencing; van Oudenaarden and Kerkhoven as experts in single cell sequencing; Holstege as expert in transcriptomics; and Brummelkamp, Beijersbergen and van den Heuvel as expert in various genetic approaches.

- **Proteomics and protein mass spectrometry** refers to the application of mass spectrometry to the study of proteins. This includes proteomics, the large-scale study of proteins, particularly their structures and functions and native protein mass spectrometry, the study of intact proteins using mass spectrometry. Bioscopy includes Heck, Altelaar and Lemeer as experts in proteomics and protein mass spectrometry.

- **Glycomics** is the comprehensive study of glycomes (the entire complement of sugars, whether free or present in more complex molecules of an organism), including genetic, physiologic, pathologic, and other aspects. Complex sugars molecules (glycans) decorate every cell of every living organism and are involved in a wide range of biological and disease processes. Bioscopy includes Boons and Heck as experts in glycomics.

- **Lipidomics** involves the identification and quantification of the complete lipid profile within a cell, tissue or organism and allows the large-scale study of pathways and networks of cellular lipids in biological systems. Bioscopy includes Helms as expert in lipidomics.

- **Metabolomics** represents the study of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. Bioscopy includes Berkers and Burgering as expert in metabolomics.

- **Integrated biomolecular modeling** aims to deliver accurate three dimensional structural models of biomolecules, their dynamics and their interactions with other molecules using a variety of experimental and bioinformatics data. Bioscopy includes Bonvin, Perrakis and Joosten as experts in molecular modeling.

- **Bioinformatics** is an interdisciplinary field that develops methods and software tools for understanding biological data. As an interdisciplinary field of science, bioinformatics combines computer science, statistics, mathematics, and engineering for the analysis, integration and interpretation of large-scale data collections resulting from systematic multi-level and longitudinal measurements as well as integration with public database knowledge. Bioscopy includes Bonvin, van Breukelen, Cuppen, van Kemmeren and Snel as experts in bioinformatics.

- **Systems biology** is a biology-based inter-disciplinary field of study that focuses on complex interactions within biological systems, using a holistic approach. Bioscopy includes Snel and de Boer as experts in the computational and mathematical modeling of system wide data generated by –omics guided experiments in complex biological systems.

Technical case & Uitdagingen en risico's (gecombineerd antwoord)
– Geef op hoofdlijnen een technische beschrijving van de faciliteit. Hoe zit de faciliteit in elkaar en hoe werkt het? – Beschrijf welke onderdelen/technieken beproefd zijn en welke geheel of gedeeltelijk nieuw? – Beschrijf de belangrijkste technische knelpunten en geef aan hoe deze opgelost zouden kunnen worden. – Beschrijf de belangrijkste risico's.

To achieve the next steps in understanding molecular and cellular biology, Bioscopy focuses on achieving breakthroughs at three levels of cellular complexity, the level of the structures of biomolecules (structural biology and bioimaging), the level of composition and interactions between biomolecules (omics) and the system-wide level (systems biology).

1. Merging of structural biology with bioimaging

Bioscopy will determine the molecular structures and dynamics of large molecular machines, such as large protein complexes, protein-nucleic acid or protein-lipid complexes, and of molecular machines embedded in the cellular membranes. Technologies used to study this level include X-ray crystallography to determine very high resolution atomic models of the biomolecules, NMR spectroscopy to study molecular structure and dynamics in cells at atomic resolution, electron microscopy to determine large and supra-structures inside cells and organelles, protein mass spectrometry to analyze the composition and topology of the complexes and a combination of electron microscopy and light/fluorescence microscopy including functional imaging and super-resolution microscopy to study their rapid conformational changes, molecular interactions and controlled movement through living cells. The results from those various techniques will be integrated through computational biomolecular modelling, bioinformatics and big data analysis. In each of these technologies, important next steps in technology will have to be developed and in all cases, participants in Bioscopy are at the forefront of these developments:

- As **X-ray crystallography** relies on the generation of crystals from biomolecules, the proteins of interest need to be expressed and purified prior to crystallization. The analysis of truly cellular complexes entails methods that allow "real" protein complexes to be isolated directly from cells and crystallized. To achieve this we need methods that allow obtaining enough material to reliably generate crystals, as the natural heterogeneity of protein complexes in cells means many different forms of a complex can exist. Integrating

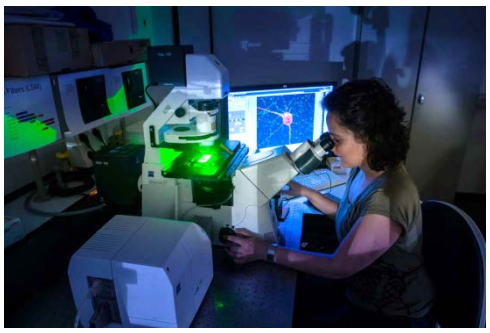
the process of obtaining homogenous complexes with genetic and proteomics approaches that could allow to define the exact components and post-translational modification status of macromolecular machines is key.

- **NMR spectroscopy,** an established technique for studying the structure and dynamics of macromolecules in solution, has already made the first small steps towards obtaining the structure of biomolecules directly from inside living cells, but a significant effort is required to optimize this technology and achieve atomic resolution of proteins. Recent investments in the Netherlands in NMR instrumentation using ultra-high field magnets are crucial to this development. The bottleneck here will be achieving sufficient sensitivity and specificity to be able to obtain the in vivo structures of particular molecules, but recent work using so-called DNP (dynamic nuclear polarization) technology has provided proof-of-principle for this.

- Recent technological innovations in **Electron Microscopy (EM)** are causing a 'resolution revolution' in life sciences. The development of direct electron detectors and novel image processing methods are propelling EM as new key method for molecular structural biology. The revolution on obtaining structural models of macromolecular complexes has already been victorious, as acknowledged by announcing single particle EM the method of the year 2015. EM is also in particular suitable to determine structures of molecules inside cells, and groundbreaking developments in what is called Electron Tomography will be crucial in this respect. Methods are being developed that allow the electron microscope to look inside cells, by removing the outer parts of a cells using a so-called focused ion beam. This technology is very new and major efforts will be required to turn this into a reliable method and allow for proper processing of all data.

- **Correlative light-electron microscopy (CLEM)** integrates EM with light and fluorescence microscopy. Combining the strength of both technologies on the same sample results in an advanced imaging tool that surpasses the capacity of either method alone. EM reveals structural details that cannot be seen by regular microscopy, while light and fluorescence microscopy provide the direct connection to functional assays and live cell imaging not possible by EM and facilitate navigation through the sample. CLEM on sections of fixed cells (sectionCLEM) is a highly powerful tool to interpret fluorescent protein localization patterns in e.g. pathological conditions, after drug treatment or gene knockdown. Correlation between live cells and EM (live cell CLEM) is a unique method to convey dynamic parameters to high-resolution images. Major challenges are developing probes and approaches for optimal resolution by light and electron microscopy.

- **Light microscopy**, including different fluorescence microscopy techniques that allow measuring the conformations and behavior of cellular structures and individual molecular components of these structures, down to single molecule level, in living cells and organisms in real time. This includes the continuously improving super-resolution microscopy, which will allow bridging the gap between cell biology and structural biology. Fluorescence microscopy has the potential to provide direct information on the localization, conformation, dynamics, and functional interactions between biological molecules in their natural environment. A major challenge is live imaging of molecules at endogenous concentrations in three-dimensional samples at a super-resolution level without incurring significant toxicity. Important areas where rapid progress is expected in the future are the development of novel smart probes, improved illumination schemes (such as selective plane illumination or employment of adaptive optics) and faster cameras.



Inside living cells

An important feature of the technologies described above is the shift of focus from the descriptive analysis of the structure and location of biomolecules to studies aimed at determining the **functionality and activity of molecules at specific sites inside cells and whole organisms**. This shift is enabled by the strongly improved possibilities of genome modification (for example, the so-called CRISPR/Cas9 genome editing technology which allows easy high-throughput gene modification of human cultured cells and model organisms to incorporate specific probes into endogenous gene products) and improved tissue culture approaches (such as use of patient-derived tissue organoids to study diseased cell states using cognate models).

2. Increased sensitivity and integration of "omics" technologies

Bioscopy will map the dynamically changing interactions between a variety of biomolecules inside cells, by improving, combining and integrating information on a large number of different molecular building blocks such as DNA (genomics), RNA (transcriptomics), lipids (lipidomics), sugars (glycomics), metabolites (metabolomics) and proteins (proteomics). In each of these technologies, important next steps in technology will have to be developed and in all cases, participants in Bioscopy are at the forefront of these developments:

- **Next-generation sequencing technology** already has made it possible to cost-effectively determine the complete genetic makeup of a system (**genomics**), allowing the accurate determination of all protein building blocks and isoforms thereof. In addition, RNA-seq provides a sensitive (indirect) measure of abundances of RNA molecules (**transcriptomics**), which is required for quantitative modelling. Furthermore, a broad range of methods have been developed to measure DNA-DNA and DNA-protein interactions. Future developments will include optimization of these techniques for sensitive and highly parallel measurements in individual cells. Emerging **single molecule sequencing** technologies are expected to have a major impact in this respect. Single cell transcriptomics, currently only in the early stages of technology development, will make pivotal contributions to our understanding of cell types, differentiation- and developmental programs, as well as to studying cellular heterogeneity in diseases such as cancer. It is anticipated that the development of improved calibration tools within RNA-seq, and higher throughput single molecule RNA microscopy, will result in transcriptome quantification on a per cell basis, pivotal for advanced modeling of cellular processes.

- **Proteomics and protein mass spectrometry** plays a pivotal role in cellular characterization by allowing detection of nearly all proteins expressed in a cell, tissue or body fluid through proteomics and the partial structural and functional characterization of proteins through native mass spectrometry. The functional understanding of the cellular proteome requires the analysis of the occurrence and dynamics of protein-protein interactions and protein post-translational modifications regulating protein function. Recent success with measuring protein interactions using native- and cross-linking mass spectrometry and measuring a plethora of post-translational modifications using proteomics provide strong proof-of-principle that mass spectrometry is ideally suited to bridge the gap between –omics/system-wide analysis and in-depth structural biology studies, a central aim in Bioscopy.

- The challenges in **metabolomics** arise from the fact that the diverse chemical nature of metabolites precludes at present the use of a "one-size fits all" technology to measure the complete spectrum of metabolites present in a biological sample. Future developments to circumvent those issues include for example the development of Direct Infusion Mass Spectrometry for metabolomics to measure a large number of metabolites rapidly and simultaneously. To achieve single cell resolution, microscaling of sample preparation is required (e.g. through the use of microfluidics) and increasing sensitivity of mass-spectrometry. An alternative approach being developed is imaging technology with metabolic sensors directed to specific metabolites to study metabolism at the single cell level in a spatiotemporal manner.

- **Lipidomics** analysis constitutes the detailed analysis and global characterization, both spatial and temporal, of the structure and function of lipids (the lipidome) within a living system. Lipidomic technology is only at the beginning of its development and next generation mass spectrometers operate at unprecedented speed, accuracy, and sensitivity. Recent work shows the feasibility and possibilities of high-throughput lipidomic analyses, allowing for the first time integration of lipidomics with other (omics) techniques. In addition, lipidomic analysis of single bovine oocytes has been accomplished, showing that single cell analysis is within reach in combination with improved lipidomic techniques.

- **Glycomics** is an emerging field of integrated research to study structure–function relationships of complex glycans. Studying these biomolecules is still very challenging. In the Netherlands, there is currently a lack of infrastructure to analyze, synthesize the structures of complex glycans and glycoconjugates, and to examine biomolecular interactions by technologies such as glycan arrays. It is clear that to fully realize integrated omics, infrastructure will need to be established to decipher the complexity and functions of glycome.

Data integration

With the rise of novel -omics technologies and through large-scale consortia projects, biological systems are being further investigated at an unprecedented scale, generating large datasets. The interpretation of these datasets is often complicated by the heterogeneity of starting material that is being analyzed. Furthermore, integration of these -omics technologies constitutes not only a conceptual challenge but a practical hurdle in the daily analysis of -omics data. Proper (omics) data integration needs careful planning ahead of the experiments. Experimental design is therefore an important step, often done in collaboration between experimentalists and bioinformaticians. Bioscopy will develop methods that allows for omics data integration, and support scientists in their experimental design to be able to make the most use of the data generated.

Currently, already many efforts focus on the integration of different methodologies, by combining multiple technologies in single projects. However, different technologies often require different experimental designs and the requirements are not always compatible between different technologies and the data obtained can be heterogeneous due to differences in sensitivity and completeness between technologies. Bioinformatics plays a pivotal role in experimental design and subsequent data analysis. To date most experimental techniques produce a single type of data, e.g. DNA sequences and abundances from next-generation sequencing technology and protein sequences and abundances from proteomics. On top of that, each technique needs specific bioinformatics tools to enable scientists to explore their data. However, by clever design of an experiment that covers many layers of data, from a multitude of experimental techniques, it becomes possible to better integrate the data at a much earlier phase. Allowing for a more comprehensive an in depth analysis of the data at all experimental levels.

In this respect, data from structural biology and imaging can even be combined with next-generation sequencing technology, proteomics and data from other techniques, as long as the data is from a single well-controlled source and experiment. Bioinformaticians, biostatisticians and experimentalist therefore have to design, together, a complete experiment *a priori*. Bioscopy will play an important role in bringing together the bioinformatics experts to facilitate this part of the experimental design.

3. Connecting different data streams through bioinformatics and systems biology approaches

In addition to the specific role of bioinformatics in bringing specific technologies together, we envisage a much broader role for bioinformatics in the interpretation of the data obtained in Bioscopy, the integration of all the information from the various technologies in system-wide mathematical models and in ensuring the proper annotation and storage of all data generated. A major challenge will be to validate complex interactions in the cellular environment and also be able to experimentally suggest novel complex interactions between biomolecules: this bring into play the need to integrate data from structural methods with not only imaging technologies but also with genetic approaches that allow to find and validate interaction networks.

Molecular modeling

Current-day molecular modeling approaches offer a manner to integrate the multitude and variety of experimental data that Bioscopy will generate in order to add the structural dimension to the systems studies. As an example, the HADDOCK tool (developed by Bonvin) can handle a variety of experimental data originating from Nuclear Magnetic Resonance, Cryo-electron microscopy, mass spectrometry, FRET (Fluorescence resonance energy transfer) to name a few, and any experimental method capable of providing interface, shape or distance information about the molecules interacting. In particular the integration of NMR data with X-ray crystallography, microscopy or mass spectrometry for the first time would allow obtaining comprehensive views of biological systems across different time and length scales. This combined used of data originating from various techniques to model structure, dynamics and interactions of the systems under study will require novel, integrative approaches to handle and balance properly various types of information without introducing biases in the results (e.g. following Bayesian approaches). As another example, we anticipate the increased, renewed use of existing data for integrated purposes. Work of the Perrakis/Joosten group already allows to continuously adjust the quality of existing macromolecular structures in the Protein Data Bank (PDB) to reflect the most recent advances, bringing new life to decades-old models. A major goal here will be to migrate the extensive knowledge on making X-ray crystallography to single particle EM, which is revolutionizing the filed.

Bioscopy will catalyze these processes by bringing together experimentalists, computational and bioinformatics experts. With such studies, biological processes and the cellular response to external alterations can be directly and comprehensively studied in pharmacology and medicinal chemistry but also during biocatalysis and in the context of plant biotechnology or food science.

Data storage and computing power

Computing and storage needs will increase. In particular dealing with an enormous amount of data will cause a challenge to the computing since data can no longer be moved easily. Solutions will have to be developed to bring the computing to the data rather than the data to the computing. The best-suited e-Science solutions will be optimally combined to serve the needs of Bioscopy. These will include, High Performance Computing, High-Throughput Computing and Cloud Computing solutions integrated with big data solutions for storage, analysis and visualisation. For reaching the bioinformatics and computing needs, Bioscopy will closely interact with the relevant national infrastructure and expertise provided by the Dutch SURFSara, but also by international initiatives such as PRACE for supercomputing, the EGI for distributed computing and EUDAT for data sharing and storage. The major challenges to overcome are dealing with the increasing data volumes, automated annotation and searching of complex data (such as microscopy data) and informative integration of multiscale data.

Systems biology

If compatible data is generated by different technologies, data integration from the various analytical scales into models will allow the development of a system-wide, high resolution, quantitative, dynamic view of molecules inside living cells. Computational structural modeling, supported by high-end high performance computing will be used to integrate information from different technologies to achieve high resolution information on the structure and dynamics of molecular machines inside cells. Integration of information from the biomolecular

interaction mapping will generate system-wide interaction network of the biomolecules, including quantitative parameters such as concentrations and affinities. Automated image analysis and computational correlation of imaging data from different technologies will yield new approaches for bioimaging of molecules and complex inside cells. This will require improvements at the level of the technologies to ensure the generation of compatible data, but also a significant bioinformatics effort concerning data warehousing and integrity, to be able to maintain and store all this data in a sustainable way, in line with current-day guidelines for the storage of scientific data.

Feasibility

Because of the overarching and distributed nature of Bioscopy, the infrastructure has a high chance of being developed successfully. Bioscopy will be a superordinate infrastructure that will not replace the existing facilities, but will serve as the main hub for development and access to *integrated* technologies. Since these developments will take place between many different technologies, the risk is distributed and there is no single point of failure for the development of the facility. Importantly, experience with setting up large and integrated facilities is abundantly available amongst the partners due to the links with for example the Dutch Tech Centre for Life Sciences (DTL), Instruct-NL and NL-Bioimaging AM (see below), meaning that all relevant expertise for a successful development of Bioscopy is present in the consortium. A small risk is the fact that, since Bioscopy only covers the integrated technology development and access, Bioscopy is dependent on continued funding of the participating facilities, through universities, NWO and the European Commission. However, this risk is limited, because the facilities are well embedded in the Dutch scientific landscape, are crucial in current-day molecular and cellular life science and have an excellent track record of sustainable operation.

B. INBEDDING
Hoe past de faciliteit in het (internationale) landschap van grote onderzoeksfaciliteiten?
– Hoe wordt de nationale toegang gegarandeerd?

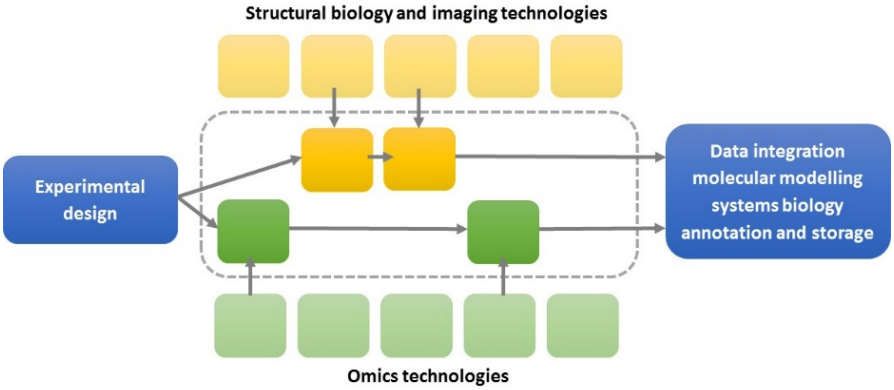
Bioscopy will consist of two major efforts, on the one hand a development programme to generate the novel integrated technologies by expert scientists (phase II, see below), and on the other hand integrated access to a multitude of existing platforms as well as the novel expertise at the participating institutions (phase III, see below).

At the technology development side, the strategy is to strengthen the existing groups with the world-leading profile in particular technologies, invest in young investigators developing cutting-edge methodologies and strongly promote well-structured interactions between these groups to increase growth in interdisciplinary areas and merging of technologies, for example, by hiring junior researchers for collaborative activities between groups.

The support for access to the facilities for external scientists will comprise several steps:

1. Access to the infrastructure will be provided through a website where interested users can submit proposals for integrated access to the facilities. This application system will be modeled after experience that has been obtained with similar systems by various participating facilities. Proposals will be required to requested access to integrated projects, involving either multiple technologies (structural biology and imaging and/or omics) to be integrated at the data level, or to the novel integrated technologies developed in Bioscopy.
2. After submission, the proposal will be reviewed by the operators of the requested facilities for technical feasibility, by bioinformaticians for experimental design and data integration and by independent researchers for scientific merit.

3. A positive technical and scientific review will be required for the proposal to be executed. The bioinformatic review can lead to suggested modifications in the experimental setup to ensure suitability of the project and the expected results with data integration strategies.
4. Upon acceptance of the proposal, the researcher will be invited to discuss the requested integrated project with relevant Bioscopy representatives. When appropriate, adjustments to experimental design will be implemented to improve data integration between technologies.
5. After finalization of the research project, execution of the projects will be the task of local operators at the participating facilities.
6. Following data acquisition, the scientist will be supported in the analysis of the data by Bioscopy bioinformaticians in the analysis of the results (including molecular modelling and system biology where relevant) and the selected strategies for proper integration, annotation and storage of the data obtained.



Schematic overview of how different Bioscopy components together would enable integrated research projects for users of the infrastructure.

The *currently existing* facilities which will form the basis of the Bioscopy infrastructure to which (integrated) access will be provided are described below. The initial organization of Bioscopy during Phase I of the project will tap extensively into the existing technical and scientific expertise of these facilities as well as into the expertise of, amongst others, DTL, Instruct-NL and NL-BioImaging AM in how integrated development and access is best organized. Continued (financial) support for these facilities and initiatives (as decribed above) is therefore crucial for the success of Bioscopy, which will form the overarching entry point for development of and access to integrated technologies.

Structural Biology & Bioimaging

Biology Imaging Center (Utrecht University; Akhmanova)
The Biology Imaging Center (BIC) of Utrecht University provides access, support and training in advanced light and fluorescent microscopy techniques. The combination of single molecule biophysics and strong cellular expertise, which makes it possible to perform analysis of the same molecules in vitro and in vivo, is unique. Available technologies include Phase Contrast microscopy, VE-DIC microscopy, regular Wide-Field Epifluorescence microscopy, Total Internal Reflection Fluorescence (TIRF) microscopy, Laser Scanning and Spinning Disk Confocal microscopy, Spinning Disk and Two-Photon microscopy, Fluorescence Recovery after Photobleaching (FRAP), photoactivation, photoablation, super-resolution localisation microscopy (PALM/STORM) and Stimulated Emission Depletion (STED) microscopy.

Cell Microscopy Core (University Medical Centre Utrecht; Klumperman)
The Cell Microscopy Core (CMC) comprises equipment for fluorescent microscopy, live cell imaging and transmission electron microscopy. Together this comprises the full range of microscopy methods for studies at the subcellular, cellular and tissue level. In addition, the CMC is specialized in immuno-electron microscopy, correlative light - electron microscopy (integrating live cell imaging or light microscopy with electron microscopy on a single sample) and 3-dimensional electron microscopy.

Cell Observatory (Leiden University; Koster)
The Cell Observatory at Leiden University houses cutting-edge bio imaging technology and other facilities, aimed at visualizing the dynamic structures of life - from molecule to cell. Facilities include atomic force microscopy, electron microscopy, light microscopy, flow cytometry, X-ray diffraction and electromagnetic spectrometry.

Center of Cellular Imaging (Utrecht University; Stoorvogel)
The Center of Cellular Imaging (CCI) provides access for researchers from UU, UMCU and industry to fluorescence microscopic techniques, including wide-field epifluorescence microscopy, confocal fluorescence microscopy, total internal reflection fluorescence microscopy (TIRF), fluorescence recovery after photobleaching (FRAP, photoactivation, photoablation, two-photon microscopy, super-resolution localisation microscopy (PALM/STORM)/single molecule light microscopy (SMLM), and structure illumination microscopy (SIM).

Electron microscopy square (Utrecht University; Förster)
The EM Square at Utrecht University is devoted to the development and application of technologies for (cryo) specimen preparation, 3D-(cryo) electron microscopy data collection, as well as image analysis for cell biology and material sciences applications. The infrastructure includes state-of-the-art Transmission Electron Microscopes (TEMs) and Scanning Electron Microscopes (SEM), with a full range of possibilities such as STEM, EDX and energy-filtering. In addition it has a specialized focused ion beam SEM (FIB-SEM).

Leeuwenhoek Centre for Advanced Microscopy (LCAM) (University of Amsterdam, Amsterdam Medical Centre (AMC) and Netherlands Cancer Institute; Gadella and Jalink)
LCAM is a collaborative expertise centre in advanced microscopy of the UvA science and medical faculty and the Netherlands Cancer Institute. LCAM harbors a full range of advanced fluorescence microscopy instrumentation: confocal (point scanning slit scanning and spinning disk), multiphoton, wide-field, TIRF, PALM, STORM, GS-DIM, FLIM, FCS, FCCS, RISC & other image correlation techniques, FRET, FRAP, , super-resolution, high content screening (96 well screening), photo-activation (uncaging, photoswitching and optogenetics) fully embedded in molecular/cellular human life sciences. LCAM was recently ratified as Flagship 'node' for Functional Imaging in the Euro-BioImaging ESFRI consortium. Functional Imaging is the integrated quantitative fluorescence microscopy, spectroscopy and probe/bio-sensor development to obtain direct molecular mechanistic information (kinetics, localization dynamics, (anomalous) diffusion, interaction, conformation, conformation dynamics, second messenger levels, enzyme activity) from single living cells and multicellular systems.

Nanobody facility (Utrecht University; van Bergen en Henegouwen)
Nanobodies that bind to any possible target can be selected using phage display technology. The facility is experienced in further functionalization of the nanobodies using different conjugation strategies including sortase tagging and click-chemistry. Examples exist for application of different nanobody conjugates for superresolution light microcopy, electron microcopy, and for development of novel diagnostic and therapeutic approaches including targeted nanoparticles and photodynamic therapy.

National single crystal X-ray facility (Utrecht University; Gros)
The National single crystal X-ray facility at Utrecht University provides services for the determination of structures of small molecules and proteins using X-ray crystallography. Examples of structural studies include model systems that mimic catalytic sites in proteins or synthetic catalysts to be used in the clean production of desired pharmaceuticals. The available equipment allows screening experiments for protein crystallization and co-crystallization and soaking of pharmaceutical fragments, with in-house structure determination.

NeCEN (Leiden University; Koster)
The Netherlands Centre for Electron Nanoscopy (NeCEN) is the national research facility for high resolution cryo-electron microscopy in the Netherlands. At NeCEN two of the most advanced 300 kV cryo transmission electron microscopes (TEM) are installed and operational as excellent high-end facility.

NKI Protein facility (Netherlands Cancer Institute; Perrakis)
The NKI Protein Facility provides support for the production, purification, characterization and crystallization of proteins. An important aspect for it is an integrated environment for studying macromolecular interactions by biophysical techniques. It is a national facility supported by NKI and NWO and is accessible researchers throughout the Netherlands, and in Europe through the iNEXT program and as part of Instruct and the Association of Resources for Biophysical Research in Europe (ARBRE). Researchers who don't have the experience or the facilities to produce and analyze proteins can request for assistance with the design and performance of the experiments.

NMR Large Scale Facility (Utrecht University; Baldus, Boelens, Bonvin)
State-of-the-art instrumentation for NMR experiments on soluble molecules or heterogeneous preparations (solid-state NMR) are available at the NMR Large Scale Facility. Currently, NMR fields range from 500 MHz to 950 MHz (solution NMR) and from 400 MHz to 950 MHz (solid-state NMR). In addition, two solid-state spectrometers (400 and 800 MHz) are equipped with gyrotrons for DNP (dynamic nuclear polarisation), which provides highly increased spectral sensitivity. The facility also functions as computational facility for NMR and structural biology consists of linux clusters with over 500 CPU cores and also provides access to GRID computing.

Omic technologies

Cell Screening Core (Utrecht University; Egan)
The Cell Screening Core at the Department of Cell Biology of the UMC Utrecht, is a well-established core for high-throughput screening. It has an automated high-throughput cell screening system in use since 2009. This platform is designed to allow biomedical researchers to screen a variety of siRNA and compound libraries in 96- and 384-well plates. Research has concentrated to a great extent on the use of high content screens that utilize automated microscopy, thus leveraging the extensive experience in fluorescence light microscopy present within the department in the form of the Cell Microscopy Core.

Genomics Core Facility (Netherlands Cancer Institute; Ron Kerkhoven)
The genomics core facility is dedicated to perform the deep sequencing experiments for users from NKI research departments. We also frequently collaborate with medical research groups. We take in cells, tissue and nucleic acids to perform library preparation and sequencing of all possible types of NGS. Expertise is available with wet-lab and bioinformatic aspects of whole genome sequencing (WGS), whole exome sequencing (WES), targeted sequencing, RNA sequencing (RNA-seq), amplicon sequencing (Ampli-seq), Chromatin Immunoprecipitation sequencing (ChIP-seq), small RNA-seq and with sequencing of short hairpin and CRISPr/Cas9 library screens. We work with fresh material as well as with FFPE material extracted from paraffin blocks. New developments comprise single cell transcriptome analysis of hundreds to thousands of cells using DropSeq, with an interest in bringing this system to the level of CNVseq and full genome sequencing in due time.

Next-generation sequencing facility (UMC Utrecht; Cuppen)
The next-generation sequencing facility is embedded within an active research environment at the Hubrecht Institute and the University Medical Centre Utrecht, aiming at the use of next-generation sequencing technology beyond the current state-of-the-art. Extensive expertise is available with wet-lab and bioinformatic aspects of whole genome sequencing (WGS), whole exome sequencing (WES), targeted sequencing, RNA sequencing (RNA-seq), amplicon sequencing (Ampli-seq), Chromatin Immunoprecipitation sequencing (ChIP-seq), and small RNA-seq and single molecule sequencing.

NKI Robotics and Screening Center (Netherlands Cancer Institute; Beijersbergen)
The NKI Robotics and Screening Center (NRSC) provides advanced technology platforms to perform large scale screening projects using cell based- or biochemical read-outs. The NRSC provides access to large collections of functional genomic screening tools including genome wide RNAi collections (siRNA and shRNA libraries) and CRISPR collections. In addition, the NRSC has a numerous small molecule collections. These screening technologies enable researchers to discover novel gene functions, to unravel molecular pathways and mechanisms, to discover novel drug targets and to support the identification of small molecules both for biological tools and novel drug leads. The NRSC provides technology and infrastructure for medium to high throughput applications and support and expertise for automated cell and non-cell based screening projects.

NPC Proteins@Work proteomics facility (Utrecht University & Netherlands Cancer Institute; Heck, Altelaar)
The NPC Proteins@Work proteomics facility provides access to state-of-the-art proteomics technology, equipment and expertise to researchers in the Life Sciences from academia and industry at its core facility at Utrecht University and at its nodes at the UMCU, NKI and the Erasmus MC. The facility is an expert center for state-of-the-art proteomics analysis that develops and implements innovative mass spectrometric methods for efficient and detailed characterization of biomolecules in relation to their biological function. The facility offers mass spectrometry based proteomics technology for applications ranging from single protein identifications to the analysis of complex, whole-cell lysate, samples and determination of posttranslational modifications.

Lipidomics Facility (Utrecht University; Helms)
The Lipidomics Facility at the faculty of Veterinary Sciences of Utrecht University houses chromatographic and mass spectrometric equipment that is specialized in the analysis of lipids. It provides complete molecular analyses of a broad spectrum of (phospho)lipids (lipid fingerprinting) in the picomole range as well as analysis of unusual lipids (lipid biomarkers). Available technologies include MS based lipidomics techniques (three mass spectrometers, all equipped with ion sources (turbo-)ESI, nano-ESI, and APCI, autosamplers, micropumps, UV detectors, UPLC and variable wavelength Fluorescence detector), assays for lipid metabolism and lipid flux using heavy isotope labeled precursors, lipid isolation and separation techniques.

Metabolomics Facility Leiden (Leiden University; Hankemeier)
The Metabolomics Facility Leiden offers a highly structured environment for advanced metabolomics studies. The facility offers state-of-the-art platforms that each cover a part of metabolism and together span the complete human metabolome. Expertise is available to support screening projects and identifying active compounds in natural products. The metabolomics platforms are based on gas chromatography, liquid chromatography and capillary electrophoresis coupled with mass spectrometry, and NMR. The facility is geared towards biomarker discovery of human disease and health and to profiling in-vitro and human and microbial cell samples.

Utrecht Bioinformatics Center (Utrecht University; Snel, van Breukelen, Kemmeren, Bonvin)
The Utrecht Bioinformatics Center is a center for bioinformatics that combines many different bioinformatics aspects and expertise, such data processing, analysis and management of big data. More over the UBC maintains and facilitates the Utrecht High Performance Compute facility and the UBC provides training and education in bioinformatics. The UBC facilitates the integration and analysis of different data sources and also provide the necessary infrastructure to do so. The UBC also has tight connections with both national (SURFSara) and international (European Grid Initiative) computational infrastructures).

Utrecht Centre for Medical Metabolomics (University Medical Centre Utrecht; Burgering)
The Utrecht Centre for Medical Metabolomics (UCMM) is a joint initiative of the department of Molecular Cancer Research) and the department of Metabolic Diagnostics of the UMC Utrecht. It unites metabolic knowledge from both the diagnostic laboratory and more fundamental metabolic research. The UCMM is specialized in the study of metabolites in individual cells, tissues, and body fluids in context of biological perturbations and nutritional status. It has a variety of mass spectrometry setups and develops and validates targeted and untargeted assays.

– Sluit het aan op reeds bestaande faciliteiten?

Bioscopy is a distributed and overarching infrastructure encompassing many existing national and European research facilities, each of which focuses on a certain set of technologies. Many of these infrastructures are part of the NWO National Road Map Large-scale Research Infrastructures or the European roadmap of the European Strategy Forum on Research Infrastructures (ESFRI). The main goal of Bioscopy will be to achieve integration of the activities of these infrastructures, as well as the other involved technologies, thereby providing a single entry point for researchers to gain integrated access to state-of-the-art technologies. Bioscopy has, in particular, strong links with the following infrastructures and initiatives, and their coordinators are all applicants on this proposal.

Linked infrastructures on the NWO Roadmap (edition of 2012):

uNMR-NL (coordinated by Utrecht University)
NMR spectroscopy is one of the most widely applied analytical methods and has found abundant application in biology, medicine, but also outside life sciences, in fields such as materials research. This versatility led to a combined effort in which five major Dutch centers for magnetic resonance research in structural biology, materials and metabolic mapping as well as imaging techniques, together with the public private partnership for analytical chemistry TI-COAST, formed in 2011 a national consortium for NMR. This concerted effort centered around the implementation of a national ultrahigh-field Nuclear Magnetic Resonance facility (uNMR-NL) that aims at providing open access to a new generation of NMR instruments operating at ultra-high field strength across scientific disciplines and industrial research. As an important step in this direction, the uNMR-NL consortium received funding from the NWO National Roadmap for Research Infrastructures to place the first of such an ultra-high field instrument, a 1.2 GHz standard bore NMR, in the Netherlands. The first step of the development of this facility has been completed, with the installation of an operational 950 MHz instrument in 2015.

Proteins@Work (coordinated by Utrecht University)
Proteomics focuses on system-wide analysis of proteins with the goal to understand their biological function in the context of all the other biomolecules present in the cells and/or tissue. For example, it allows studying the regulatory effect of protein interactions and modifications on cancer and autoimmune diseases. The Netherlands Proteomics Centre (NPC), founded in 2003, combined research in proteomics technology with localized “research hotels” that provided access to technology alongside an integrated program to enhance and improve the use of bioinformatics in proteomics. The NPC is now firmly established in the Dutch national research community, and is also recognized internationally because of its high-quality contributions to the field. Built on this solid foundation in 2014, the national Roadmap Facility Proteins@Work was initiated and with NWO financial support the Proteins@Work proteomics facility currently provides access to a very wide range of both academic and industrial partners.

NeCEN (coordinated by Leiden University)
The Netherlands Centre for Electron Nanoscopy (NeCEN) is the national research facility for high resolution cryo-electron microscopy in the Netherlands. The instrumentation at NeCEN provides the means to resolve and characterise detailed molecular structures of life. Between 2007 and 2011 experts from eleven Dutch organizations worked closely together in a consortium to realize the NeCEN. In 2012 it opened its doors as an open access facility. At NeCEN two of the most advanced 300 kV cryo transmission electron microscopes (TEM) are installed and operational as excellent high-end facility. The infrastructure is especially valuable for applications of cryo-electron microscopy in the field of structural (cell) biology as it provides fundamental structural information at high resolution about biological processes that is critical for the development of novel strategies in prevention, diagnosis and treatment of diseases and for development of sustainable processes based on biological systems.

NL-Bioimaging AM (coordinated by University of Amsterdam)
The Netherlands traditionally has an excellent position in advanced microscopy worldwide. Netherlands-BioImaging Advanced Microscopy (NL-BioImaging AM) has brought together the national advanced microscopy community to share equipment and expertise and to increase the international visibility. This NWO Roadmap project capitalizes on this potential by generating a top-level microscopy infrastructure. The infrastructure, incorporates 19 partners, including all the major Dutch Universities, University Medical Centers and research institutes. The plan is to make the infrastructure accessible to all life sciences research groups in the Netherlands. NL-BioImaging AM is on the Dutch roadmap since 2012 and expects to be operational in 2017.

Other national infrastructures

NEMI
New technical developments are revolutionizing electron microscopy. The Netherlands Electron Microscopy Infrastructure (NEMI) is a *planned* national infrastructure that will unite the excellent EM centers in the Netherlands, with the goal of bringing together key instrumentation and expertise, and to provide access to these facilities. Establishing this national Infrastructure is urgent and necessary because of the high costs involved in the exploitation and continuous upgrading of top-end EMs. This national collaboration is required to get the Netherlands at the forefront of innovation in EM and to perform top research in life sciences, material sciences and geosciences, all disciplines with an increasingly high demand for EM.

Linked European infrastructures

Euro-Bioimaging (www.structuralbiology.eu)
Euro-BioImaging is the large-scale pan-European research infrastructure project on the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. The ESFRI research infrastructure Euro-BioImaging will provide open user access to a complete range of state-of-the-art imaging technologies in biological, molecular and medical imaging for life scientists in Europe and beyond. Euro-BioImaging will offer image data support and training for infrastructure users and providers and continuously evaluate and include new imaging technologies to ensure cutting-edge services in a sustainable manner. Euro-BioImaging is now in the interim phase, with representatives of 14 countries and EMBL as international organization working together towards the implementation of the infrastructure. The Netherlands participates in Euro-Bioimaging through the national initiative NL-Bioimaging AM.

Instruct (www.eurobioimaging.eu)
Instruct is an integrated research infrastructure for structural biology that is part of the ESFRI Roadmap since 2008. Since that time, it has evolved into a prominent platform where cutting-edge technology, leading expertise and pioneering training combine in support of outstanding science. Instruct champions an integrated approach to structural biology. It strives to refine the quality of structural biology research in Europe by contributing to and promoting new developments and methodologies. It also provides strategic leadership for structural biology policy in Europe. The Netherlands is a member of Instruct and offers the facilities at NeCEN, the NKI Protein Facility, and the NMR and proteomics facilities at Utrecht University to the European research community through the national initiative Instruct-NL.

Elixir (www.elixir-europe.org)

Elixir aims at providing a sustainable European infrastructure for biological information, supporting life science research and its translation to medicine, agriculture, bioindustries and society. It unites Europe's leading life science organizations in managing and safeguarding the massive amounts of data being generated every day by publicly funded research. It is a pan-European research infrastructure for biological information. The Netherlands is a member of Elixir through the Dutch Techcentre for Life Sciences (DTL), a public private partnership that aims to establish a world-class technology infrastructure to enable next generation life sciences research across life science sectors.

Finally, it should be noted that Bioscopy participants have coordinated or participated in many EU funded infrastructures projects from the 6th and 7th Framework programmes and the EU Horizon 2020 programmes. Examples of such projects are LipidomicNET (lipidomics, 2008-2012, Helms second largest contributor), WeNMR (molecular modelling & high throughput computing, 2010-2013, coordinated by Bonvin), PRIME-XS (proteomics, 2011-2015, coordinated by Heck) and iNEXT (2015-2019, structural biology, coordinated by Boelens).

- Zijn er voor zover bekend vergelijkbare ideeën (of al bestaande faciliteiten) in het buitenland? Zo ja, zou Nederland een aparte nationale faciliteit moeten hebben of betreft dit een internationale faciliteit op Europees of mondiaal niveau?

A truly integrated effort in the molecular and cellular life sciences at this level does not exist anywhere in the Netherlands or outside. The closest international peer for Bioscopy would be the European Molecular Biology Laboratory (EMBL), an international molecular biology research institution supported by 21 European member states. The EMBL operates from five sites: the main laboratory in Heidelberg, and outstations in Hinxton (the European Bioinformatics Institute (EBI), in England), Grenoble (France), Hamburg (Germany), and Monterotondo (near Rome). EMBL groups and laboratories perform basic research in molecular biology and molecular medicine as well as training for scientists, students and visitors. Each of the different EMBL sites has a specific research field. The EMBL-EBI is a hub for bioinformatics research and services, developing and maintaining a large number of scientific databases, which are free of charge. At Grenoble and Hamburg, research is focused on structural biology. EMBL's dedicated Mouse Biology Unit is located in Monterotondo. At the headquarters in Heidelberg, there are units in Cell Biology and Biophysics, Developmental Biology, Genome Biology and Structural and Computational Biology as well as service groups complementing the aforementioned research fields. Furthermore, large Universities in the US and in Europe (e.g. Harvard, UCSF, and Cambridge) have a broad set of facilities which will address similar challenges. However, setting up of a complete, integrated pipeline by combining the existing expertise, attracting new expertise and providing country-broad access to newest technologies has **the potential to bring the Netherlands to the forefront in this field world-wide.**

Hoe past de faciliteit bij de NL sterktes van onderzoek?

Bioscopy will build on the vast technological expertise of many existing Dutch facilities, infrastructures and research groups. Bioscopy will make use of the geographical proximity and existing strong connections between these labs, which excel in different research methodologies and therefore are in the optimal situation to start a large integrated infrastructure such as Bioscopy, a situation that is almost unique world-wide. Thereby, Bioscopy will be a logical next step for the further, integrated development of existing initiatives which are currently part of the Netherlands' Roadmap for Large-Scale Research Facilities and European infrastructures initiatives within the molecular and cellular life sciences domain.

Bioscopy is expected to collaborate closely with the public-private initiatives COAST and DTL, which to bring together universities, university medical centers, universities of applied science ('HBO'), public or private research institutes and companies, in the fields of analytical sciences and life science data integration, respectively:

COAST
As public-private partnership for Comprehensive Analytical Sciences and Technology, TI-COAST aims to advance excellence in the Topsectoren of the Dutch economy by providing pivotal analytical knowledge and instruments based on fundamental science and by ensuring transfer of analytical expertise between application areas. COAST strengthens analytical science in the Netherlands by uniting R&D, human capital and infrastructure programs. COAST advances R&D and innovation in analytical technologies, encourages cross-fertilization between analytical technologies and application areas, improves education in analytical science and provides access to research facilities and knowledge for players within and across economic Topsectors.

DTL
The Dutch Techcentre for Life Sciences (DTL) is a nationwide platform formed by an increasing number of life science partner institutions in the Netherlands. The need for DTL arose when the partners were confronted with challenges in high-throughput and data-intense research methods that clearly transcended individual capacities. In DTL the partners can collectively and effectively coordinate the approaches to deal with all aspects of data stewardship and analysis, as well as expensive high-end technologies, both in national and international context.

Companies
Close collaboration with specific companies that develop instrumentation is expected. Many of the partners already collaborate with instrument developers and for the development of some of the new technologies, such close collaborations will be crucial. Current collaborations between Bioscopy partners and companies include for example FEI (Eindhoven, the Netherlands) in the field of electron microscopy, MS-Vision (Almere, the Netherlands) and Thermo Fisher Scientific (Bremen, Germany) in the field of mass spectrometry, Bruker (Karlsruhe, Germany) in the field of NMR spectroscopy, Leica (Wetzlar, Germany) on STED microscopy, Nikon (Tokio, Japan) on Super Resolution microscopy. In addition, pharmaceutical companies such as Crucell (Leiden), Genmab (Utrecht) and Synthon (Nijmegen) are already familiar with the capabilities of the individual technologies, as they already collaborate with Bioscopy partners and are therefore expected to be interested to become users of the infrastructure.

Beschrijf de voordelen/belang voor NL indien zo'n faciliteit zou worden gerealiseerd. Dit mogen zowel wetenschappelijke als economische of maatschappelijke voordelen zijn.

Through coordination of a broad set of disciplines, Bioscopy will ensure optimal, integrated, use of the available infrastructural resources in the molecular life science field in the Netherlands. Bioscopy will also provide a concerted effort for developing new technologies, and through these technological developments, will provide possibilities for collaboration with national and international industrial partners, as described above.

Bioscopy will enable addressing fundamental and applied research questions on the National Science Agenda ("*Nationale Wetenschapsagenda*"), such as understanding the cause of diseases and development of improved therapies, or producing improved crops. At the same time, it will provide excellent training opportunities for young scientists and will increase the international impact and visibility of the Netherlands in life sciences, will make the Netherlands and participating institutions more attractive for young talented researchers due to accessibility of an exceptionally strong integrated infrastructure.

C. ORGANISATIE en FINANCIËN
Organisatie
Geef aan welke partijen/expertise nodig zijn voor de ontwikkeling van deze faciliteit. Geef ook aan of en zo ja hoe deze al zijn betrokken.

Scientists from the following institutions are involved in Bioscopy. These scientists all operate at the interface of technology development and applications, and most of them have expertise in the provision of access to their technologies to external scientists, through the facilities described above.

Utrecht University & University Medical Center Utrecht
Life Science is one of the strategic themes of *Utrecht University*. At the Faculty of Science, scientists in the theme Science for Life explore the fundamentals of life through high-level research in the biosciences, aiming at future solutions for health and environmental challenges. Science for Life represents the collaboration of internationally leading scientists from the Faculty of Sciences at Utrecht University, combining forces of over 20 research groups in the fields of molecular, cellular and pharmaceutical sciences, as well as in plant biology. The faculty of Veterinary Medicine performs fundamental and strategic research focused on health, disease and welfare of animals and on related public and environmental health aspects. The *University Medical Center Utrecht* strives to achieve the highest levels in, patient care, scientific research and education. It combines state-of-the-art healthcare with scientific research in multiple programmes and research centers. At the University Medical Center Utrecht, the Center for Molecular Medicine focuses on understanding the molecular basis of disease. Recently, the Prinses Maxima Center for child cancer was established in a collaboration between the University Medical Center Utrecht, the Hubrecht Institute and the Netherlands Cancer Institute.

Hubrecht Institute
The *Hubrecht Institute* for Developmental Biology and Stem Cell Research focuses on developmental biology and stem cells at the organismal, cellular, and molecular level. A variety of biological processes are being studied, mainly concerning embryonic development and development and homeostasis of organs, all at various levels, i.e. organism, cell, and DNA. In addition to basic research, the institute carries out strategic biomedical and genome research, leading to a better understanding of such diseases as cancer.

University of Amsterdam & Amsterdam Medical Center
At the Swammerdam Institute for Life Sciences of the *University of Amsterdam*, research is focused on a number of central themes. Among these themes are gene expression, the structure of DNA molecules and chromatin, protein-protein interactions, protein structure-function relations and signal transduction processes, as they take place in man, animals, plants and micro-organisms. In the domain of the human life sciences the UvA and the free university of Amsterdam (VU) join forces as part of the Science in Amsterdam initiative. To this end a brand new collaborative research building will be operational in 2016 where UvA, VU and VUMC concentrate their molecular, cellular human life sciences. The *Amsterdam Medical Center*, in close collaboration with the University of Amsterdam, performs research in the fields of cardiovascular disease, infection and immunity and metabolic diseases, all in tight a cooperation between medical doctors, biomedical researchers, biochemists, biologists, bio-informaticians and genetics and imaging experts.

Leiden University & Leiden University Medical Center
Leiden University is a broad university with a strong emphasis on research in the biomolecular sciences in its institutes of biology and chemistry. The Institute of Biology Leiden performs research in the fields of fundamental and applied microbial, plant and animal sciences. At the Leiden Institute of Chemistry, research is aimed at understanding biological processes at the molecular level to strengthen the knowledge base of human health and disease. The *Leiden University Medical Center* is an academic medical hospital and research institute focusing on top clinical and highly specialized care and patient care in combination with scientific research. The Department of Molecular Cell Biology at the LUMC aims to perform curiosity-driven basic research for the

understanding of the molecular mechanisms that underlie the function and activity of cells and tissues, the unravelling of the molecular defects that form the basis of inherited and acquired disease and to translate this knowledge and related technology into improved diagnosis or novel treatment modalities.

Netherlands Cancer Institute

The *Netherlands Cancer Institute* combines three major areas of research: fundamental, clinical and translational research. A thorough understanding of the basic processes in cells is the foundation for understanding cancerous cells. It covers all major areas of cancer research, with special emphasis on cell-based screens, mouse tumor models, cell biology, structural biology and epidemiology. The institute coordinates and participates in many clinical trials, which allows results obtained from fundamental research to be directly translated into clinical applications.

Potential additional stakeholders

In addition to these first partners, who participated in the preparation of this proposal, Bioscopy expects to identify and involve additional stakeholders, partners and/or users in Phase I of the project.

Beschrijf de (mogelijke) organisatiestructuur. Geef ook aan of er al begin van organisatievorming is.

Bioscopy will have an open management structure that will take input from a large number of external sources, to maximize the embedding of the facility in the Dutch research community. The main management body will be the Supervisory Board, in which all partners and facilities are represented. The ultimate responsibility for the execution of the project rests with this body. However, many of the responsibilities will be mandated to a central management body, the Management Board, (MB) especially with respect to the day-to-day operation and overall scientific and strategic management of Bioscopy. The Management Board will be elected by the Supervisory Board and will be representative of the various technologies and institutions represented in Bioscopy. An International Advisory Board will be composed of international renowned researchers that will advise Bioscopy on shaping, implementing and maintaining its position in research and industrial setting on the national as well as international level.
To ensure a good embedding of the facility in the Dutch and European Research Area, both Dutch Funding Agencies as well as relevant Dutch (e.g. COAST, DTL) and European initiatives (e.g. Instruct and Euro-BioImaging) will be asked to provide regular input on the operation of the facility or even participate in its operation as a full partner, when that would be beneficial for the project.

Financiering
Geef een globale beschrijving van de business case. Hoe zou deze faciliteit gefinancierd kunnen worden? Ga hierbij in de drie fases: ontwikkeling, bouw en exploitatie.

In the first few years of Bioscopy, only limited funding would be required, as this first phase will be used set up a legal and governance structure and systematic interaction between all participants to develop a “roadmap” for the fully operational Bioscopy infrastructure over the next period of 10 years. During this phase also the need for participation of additional researcher and institutions in Bioscopy will explored, as mentioned above. After that initial start-up phase, funding will be required to develop (phase 2) and operate (phase 3) the infrastructure, with funding expected to be required for (for details see table above):

- technology development, including (young) scientists specialized in such developments
- investments in emerging relevant technologies
- data analysis, integration and management efforts
- exploitation costs for access to the infrastructure, including local facility managers
- interaction between all participants
- management and governance

Since no currently existing funding scheme fits with these large ambitions, and because investments through such schemes in individual technologies boosts those fields tremendously and should therefore be sustained, the only real way to establish Bioscopy and to bring the Netherlands to a new level in integrated life sciences will be through dedicated investments in the initiative by the Dutch government.

D. VERDERE ONTWIKKELING
Beschrijf wat er moet gebeuren om deze faciliteit verder te ontwikkelen. Ga in op de belangrijkste knelpunten die opgelost moeten worden.

Currently, all funding for infrastructures is either project or facility-based. Funding for individual infrastructures is obtained from schemes such as NWO Medium and NWO Groot (which only cover investments), the NWO National Road Map Large-scale Research Infrastructure (which can covers cost for investments and partially exploitation for access) or EU infrastructure projects (which covers partial exploitation costs *for international access* only). The development of the new technologies is fundamental research in nature. Such research in the Netherlands is often funded through European sources such as ERC due to the lack of national resources. Such funding only covers project-specific needs and does not allow for providing access.

Although the participants in Bioscopy have been very successful in obtaining funding from such schemes in the past, this leads to a situation where it is very difficult to obtain funding for developing interdisciplinary facilities, the main goal of which is to connect different methodologies. Only through a concerted investment effort would it be possible to setup a truly integrative imitative such as Bioscopy. Thus national vision and funding is required to create a nationally accessible facility.

Finally, the recruitment of young experts in particular for data analysis and integration projects is difficult, due to the shortage of researchers with the background in quantitative biology. One possible solution is in house training of experts by setting up appropriate educational programs (such as specialized Master tracks), which will require a solid scientific basis at the participating institutions. The establishment of Bioscopy would be an excellent trigger to establish also a national vision on the education of experts in integrative cellular and structural biology.

Geef aan wat de ontwikkeltermijn voor deze faciliteit ongeveer zou kunnen zijn.

The first steps to establish Bioscopy can take place already in the coming few years, through targeted, yet still relatively limited funding to set up an appropriate legal structure, a governance structure and systematic interaction between all participants. The aim of these first steps would be the development of a “roadmap” for the fully operational Bioscopy infrastructure over the period of 10 years. Because developments in many of the participating fields go very fast, this step would also involve a close monitoring of these development and adjust the Bioscopy infrastructure continuously to include the latest development and remain at the forefront of science. Due to the distributed nature of the infrastructure, such new developments can easily be integrated in Bioscopy.

The next steps will be those towards an operational infrastructure and will require targeted support (see also the *Business case*) for technology development and attracting (young) scientists specialized in such developments, timely investments in emerging relevant technologies to be at the international forefront internationally, centralized planning and setting up data analysis, integration and management efforts, supporting the exploitation costs for national access to the Bioscopy infrastructure, to sustain the efforts in interaction between all participants and for the efficient management and governance of the infrastructure.

Because its strong basis in the Dutch molecular and cellular life science community, the establishment of Bioscopy can revolutionize this research field and give the Netherlands in a leading position world-wide.