NeuroPhotonics Institute

Illuminating the brain from synapse to system

Acroniem	NPI
Naam van de infrastructuur	**NeuroPhotonics Institute**

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SUMMARY

Understanding how the brain orchestrates our thoughts and actions and how it is affected during disease are arguably among the most important and challenging questions facing science today. The past decade has seen a wave of breakthroughs in light-based imaging and stimulation techniques, enabling the near real-time probing of brain activity at high spatial and temporal resolution. Nevertheless, the current light-based techniques are still insufficient to answer many fundamental questions about brain function. Harnessing the full potential of photonics research in the Netherlands and developing innovative light-based approaches in the coming decades requires the appropriate infrastructure to foster collaborative interactions between neurosciences and physics. We therefore propose to establish a multi-disciplinary NeuroPhotonics Institute to develop and apply novel light-based technologies enabling to look deeper and faster into the brain, from the level of synapses to whole systems.

At the core of the institute will be a collection of leading edge optical stimulation and imaging facilities that will be accessible for external users. In addition, the institute will host several dedicated research groups that will continue to push the boundaries of photonics development and application and ensure that the core facilities represent the leading edge of photonics research. To allow rapid carryover from photonics research to applications, the institute will also have various in-house support facilities, such as a mechanical workshop, electronics workshop, IT/data management support facility and an animal facility. Given that light-based technologies and applications are expected to remain in rapid development for many decades, incorporating a number of research groups that develop and apply new imaging paradigms will be the best way to ensure the facility remains state-of-the-art. Ultimately, the large research facility should provide capabilities that bridge the current gap between cellular studies and MRI-based tissue studies, making it tractable to answer the major questions about the brain in both health and disease.

A. SCIENCE AND TECHNICAL CASE

We propose to create a new research facility that will merge two scientific disciplines, neuroscience and photonics, with the goal to achieve breakthroughs in both disciplines through collaboration. Within the institute, physics and biology research groups will join forces to develop and apply novel light-based methods that will bridge the gap between existing cellular (i.e. microscopy) and tissue techniques (i.e. MRI) and thereby allow studying brain structure and function from a completely new perspective. Combining these fields of research in one center is essential to bridge the gaps that exist between fundamental photonics, advanced microscopy and fundamental and applied neuroscience and to establish a multidisciplinary facility with pioneering equipment accessible for the Dutch scientific community. These developments will be important to maintain the competitiveness of the Dutch neuroscience and (bio)photonics community, as several multidisciplinary centers are currently emerging in other countries (Janelia Farm Research Campus, USA; IINS Bordeaux, France).

In this section, we will outline the scientific and technical case of the proposed facility. To develop the scientific case, we will first describe important current and expected developments in neuroscience and biophotonics and discuss the expected benefits of establishing a dedicated facility to develop and apply novel photonics technology to neuroscience problems. This will be followed by a detailed description of the structure of the facility, the technical case.

SCIENTIFIC CASE

CHALLENGES IN NEUROSCIENCE

The brain confers on us all abilities that make us human. It drives our emotions, thoughts, and memories and orchestrates our behavior and actions. How these complex tasks are executed and how the large number of brain diseases should be treated are among the most pressing questions facing Dutch life sciences and society today[1]. While during the last decades neuroscience has undergone major advancements, studying the brain still remains challenging. The scientific tasks that the discipline is faced with are exceeding by far those confronting the Human Genome project in the last century. The Human Genome project was eventually made tractable by financially supporting large multidisciplinary collaborative teams to share knowledge and research tools. In recent years, neurosciences has seen similar large 'big science' initiatives funded with billions of dollars both in the EU[2] and the USA[3]. These initiatives are slowly changing the structure and practices of neuroscience and move the discipline from small-scale laboratory-based research into larger cross-institutional collaborative programs aimed to answer the key questions: how does the brain learn and how do aging, drugs and psychiatric diseases interfere with proper brain functioning?

One of the reasons we still know very little about the brain is that its underlying cellular functions are exquisitely complex distributed in both time and space, spanning many orders of magnitudes. Experimentally measuring these features simultaneously and with sufficient precision is an extremely challenging task; in the brain of humans more than 80 trillion contact points (synapses) connect the 80 billion individual excitable cells (neurons) in a dynamic wiring circuit. Each of these neurons produces intrinsic electrical activity to store and distribute information. The electrical events in an individual neuron span about three orders of magnitude; from the synaptic potentials of hundred micro-volts (~0.1 mV) to the large amplitude action potential events of one hundred millivolt (~100 mV). Electrical events occur compartmentalized at the nanometer scale as well as distributed in space and time over the neuron membrane, spreading over millimeters in the receiving branches, called dendrites. Action potentials also travel up to 1 meter along the output structures called axons. A further challenge is that electrical signaling occurs with very high temporal precision at an extremely large bandwidth, from sub-millisecond temporal resolution (action potentials) to slow electro-encephalogram activity waves at a time scale of seconds. How these spatial and temporal scales are connected and orchestrated to produce the ultimate functions of the brain and drive behavior is poorly understood, as these activities are difficult to measure simultaneously. Conventionally, brain activity is recorded by means of single electrodes sensitive to electrical currents (called the patch-clamp technique[4]). While the electrode-based method currently still provides the best means to record excitability at high temporal resolution with a good signal-to-noise ratio, it is obviously a poor strategy to provide spatial information about networks of neuron populations that function coincidentally. Therefore, **developing new principles and methods to measure the dynamics and electrical activity of multiple neurons inside intact tissue with sufficient temporal and spatial resolution is a core challenge in contemporary neurosciences.** Similarly, access to these methods will be essential to contribute to a better understanding of the brain in health and disease.

The past decade has seen a wave of breakthroughs in novel light-based imaging and recording techniques, enabling more specific and detailed probing of brain functions. Light-based technologies are typically less invasive than patch-clamp techniques and can monitor large fields of view with a high temporal resolution. We believe that these optical methods will eventually provide the dynamic information required to probe brain activity and function. We will now highlight how novel molecular light-based probes and actuators allow monitoring neuronal activity with unprecedented specificity, followed in the

[1] Nationale wetenschapsagenda (http://www.wetenschapsagenda.nl/)
[2] Markram H (2012) The human brain project. *Sci. Am.* 306:50–55.
[3] Bargmann, C. et al. BRAIN 2025: a scientific vision. Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Working Group Report to the Advisory Committee to the Director, NIH http://braininitiative.nih.gov/pdf/BRAIN2025_508C.pdf (US National Institutes of Health, 2014).
[4] Sakmann B, Neher E (2013) Single-Channel Recording, Second Edition. New York: Springer.

next section by a discussion on current and expected developments in photonics that could allow detecting and stimulating these probes and actuators within intact brain structures.

The development of **advanced optical probes** reporting on protein activity, ion concentrations or membrane potential provides access to a wealth of important, dynamic information. These light-based techniques allow for the direct observation of molecules, genetically tagged cells, cell circuits and indeed even the whole nervous system in living animals (as demonstrated in small transparent model organisms). One important class of fluorescent probes can be used to readout electrical activity. The measured signal in the form of photon flux results from chemical conversion of the electrical signal into photons by means of a reporter, and is normally observed as a change in fluorescence detected by charge-coupled devices (CCDs), complementary metal-oxide semiconductor (CMOS) or photo-multiplier tubes (PMTs). Activity probes range from indirectly reporting intracellular calcium dyes to more direct reporters of the membrane potential (voltage-sensitive dyes, VSDs). However, to be able to compete with electrode-based techniques, an optical imaging method must be able to record the smallest possible events, without averaging, deep in intact tissue. Furthermore, understanding how a single neuron functions inside a network requires imaging methods that readout signals at both high temporal (< 1 ms) and spatial resolution (<100 nm) across large regions (>1 mm). Being able to record electrical signals accurately will be essential to understand the computations performed by neurons, but we are currently still far from this goal[5].

Another important recent technology is **optogenetics**[6], which allows for perturbation of brain circuits by activating or inhibiting genetically targeted cells with specific colors of light through the use of light-sensitive protein sensors. Whereas the original, channelrhodopsin-based, tools facilitate whole-cell control of firing activity, more recent developments also allow subcellular control of important cell biological processes, such as gene expression, enzyme activity and intracellular transport[7]. These molecular sensors are genetically introduced into specific brain cells, using viral or genetic techniques, allowing targeting a specific subpopulation of cells. Optogenetics allows manipulating the brain, and studying how the neural circuits respond to specific perturbations. Importantly, the combination of cell specificity, bimodality and breadth of implementation gives optogenetics the power to connect the different levels of the central nervous system and provide neuroscience with direct causal explanations for how the machinery of the brain drives high-level functions such as behavior and cognition. This is a tremendous advancement compared to more traditional techniques, which are limited to descriptive studies of brain tissue outside of the animal, removed from its functional context.

In summary, light-based detection and control of neurons allows studying the functional role of molecularly or genetically identified group of cells in the living brain. The complementarity of these novel strategies to traditional imaging and electrical recording techniques has already revolutionized the manner in which neuroscience research is performed today, and is worldwide being recognized as a key enabling technology for the 21st century. Nevertheless, although optical techniques are much less invasive than electrode-based recordings, the downside is they are dependent on the imaging depth in the tissue and the associated wavefront distortion when light passes through tissue. As a consequence, there exist strong tradeoffs between spatial resolution and imaging depth. **Figure 1** illustrates that resolving individual brain cells and their subcellular structures (dendrites and synapses) is currently still impossible in areas > 800 μm away from the surface and most current imaging methods are limited to the first ~0.5 mm from the surface. As a consequence, regions such as the hippocampus, an important area for learning and memory, or the thalamus, responsible for sensory integration, which are >1.5 mm under the brain surface in mice cannot be reached. **Thus, new methods that can examine neuronal function in all layers and at (sub)cellular levels are highly needed.**

[5] Scanziani M, Häusser M (2009) Electrophysiology in the age of light. *Nature* 461:930–939.
[6] Boyden ES (2015) Optogenetics and the future of neuroscience. *Nature Neuroscience* 18:1200-1201
[7] Dance A (2015) Micromanagement with light. *Nature* 528:291-294

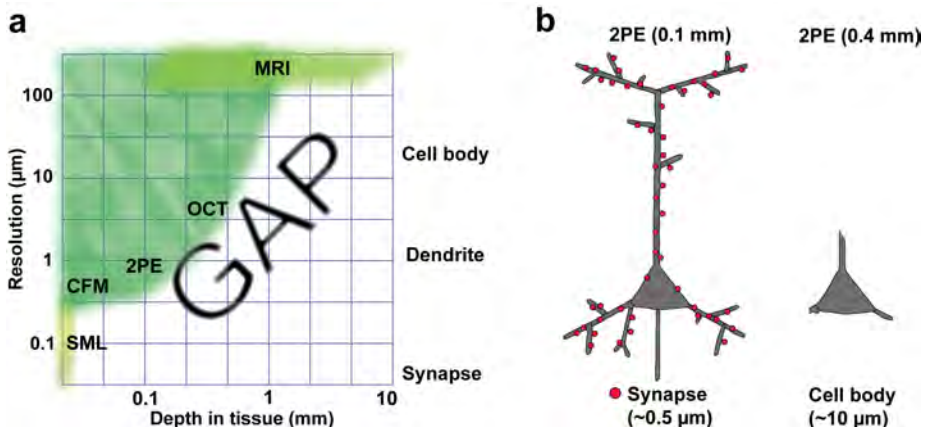


Figure 1: Chart of resolution in idealized conditions versus depth for existing optical methods in typical brain tissue. ***a)*** *Current optical methods span the green area. OCT = Optical Coherence Tomography, CFM = Confocal Fluorescence Microscopy, 2PE = 2-photon excitation, SML = Single Molecule Localization methods. Magnetic resonance imaging (MRI) and positron emission tomography (PET) are non-optical methods indicated for comparison.* ***b)*** *Schematic of spatial resolution for neuronal imaging using 2PE near the surface (left) or deep in tissue (right), failing to resolve synapses and dendrites.*

Importantly, tools to allow imaging at high spatial resolution deep into brain tissue will also have major relevance for applied research on brain disease. For example, novel experimental approaches in the field of medicine and neurosciences show that renewal and repair of the brain may be achieved by introducing induced pluripotent stem cells[8]. Stem cell-derived therapies are being pioneered for the treatment of Parkinson's disease[9] and Alzheimer's disease[10]. Understanding how stem cells integrate within the existing healthy and diseased neural circuitry is a central challenge that requires spatially precise, high-resolution imaging of targeted locations deep in the intact brain, including the substantia nigra, the primary regions where dopamine cells are disrupted in Parkinson's disease. Such photonic methods are critical for all levels of neuroscience research, from brain slices to intact brain or even the recent tools of 3D brain organoids[11].

As we will outline below, several developments in photonics and optical engineering have the potential to address these challenges and may allow deeper imaging and light delivery at higher resolution. However, it is becoming increasingly clear that the associated technical and physical aspects reach beyond what individual neuroscience groups or even neuroscience as a whole can tackle. In this proposal, we argue that only multidisciplinary research facilities can tackle these scientific tasks. Establishing a NeuroPhotonics Institute will be essential to provide the Dutch research community with the tools and infrastructure needed to maintain our position in the top rankings and to address the current important scientific and societal questions related to brain functioning[12].

[8] Clevers H, Loh KM, Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* 346:1248012.
[9] Barker RA, Drouin-Ouellet J, Parmar M (2015) Cell-based therapies for Parkinson disease—past insights and future potential. *Nat Rev Neurol* 11:492–503.
[10] Hunsberger JG, Rao M, Kurtzberg J, Bulte JWM, Atala A, LaFerla FM, Greely HT, Sawa A, Gandy S, Schneider LS, Doraiswamy PM (2015) Accelerating stem cell trials for Alzheimer's disease. *Lancet Neurol*.
[11] Lancaster MA, Renner M, Martin C-A, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA (2013) Cerebral organoids model human brain development and microcephaly. *Nature* 501:373–379.
[12] Nationale wetenschapsagenda (http://www.wetenschapsagenda.nl/)

DEVELOPMENTS IN BIOPHOTONICS

Biophotonics explores novel ways to exploit light for the study of biological matter. Optical methods are of great value in the study of biological systems due to a number of desirable properties of light:

- High resolution – Standard microscopy methods can already resolve subcellular structures,
- High sensitivity – In fluorescence methods single molecules can be detected,
- High specificity – Spectroscopic measurements or differential labelling allow to distinguish subtle genetic and molecular signals,
- Low toxicity - Long-wavelength light at moderate intensity has no detrimental effect on living cells
- Ability to activate chemical and genetic switches
- High speed – Optical signals can be acquired as fast as, or even faster than electrical signals.

However, the use of optical methods in intact living three-dimensional tissue also poses a number of challenges:

a) The resolution of standard microscopy methods is limited to half the optical wavelength, or about 200 nm, which is insufficient to resolve critical subcellular structures such as vesicles and motor proteins.
b) Optical paths are distorted by inhomogeneity of the tissue (see **Box 1**). This reduces the resolution even further especially in intact cells and tissue.
c) Light is scattered by cells, leading to a strong loss of contrast deep inside the tissue (see **Box 1**). Typically this limits cellular-resolution methods to the outer 0.5 mm.
d) Absorption of light by water leads to a loss of signal, which limits the use of long optical wavelengths to a depth of up to a few mm.

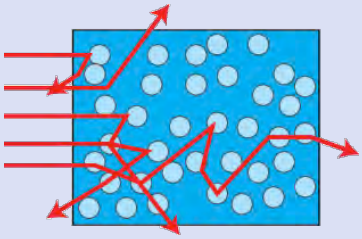
Box 1: Light in complex materials

When laser light impinges on a complex material such as tissue, it is scattered by nanoscale inhomogeneities such as the nuclei, membranes and organelles of the cells. Light transmitted through tissue typically follows a long and convoluted path incorporating multiple scattering events, as depicted in panel (a). A small fraction of the incident light continues on a straight path. The intensity of this "ballistic" light decays exponentially with depth according to the Beer-Lambert law,

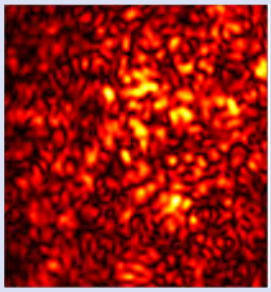
$$I(z) = I_0 e^{-\frac{z}{\ell^*}}, \qquad (1)$$

where $I(z)$ is the intensity of ballistic light at depth z, and ℓ^* is the *scattering mean free path* (also known as attenuation length or inverse scattering coefficient). Small angle scattering perturbs the flow of light less than large angle scattering. The *transport mean free path* (also known as momentum correlation length or inverse reduced scattering coefficient for lossless media) expresses the length over which the angle is completely randomized, and thereby accounts for the average scattering angle[13].

As scattering in static tissue does not perturb the phase coherence of the light, scattered waves that arrive by 'different paths' through the sample show interference. Depending on the detailed positions of the random scatterers, this interference is constructive at some positions and destructive at others. The result is a characteristic pattern of dark and light spots known as laser speckle, shown in (b). This pattern is stable as long as the scatterers inside the sample do not move and the illumination



a) Cartoon of the light paths through a strongly scattering medium. Most light is backscattered in the surface layer.

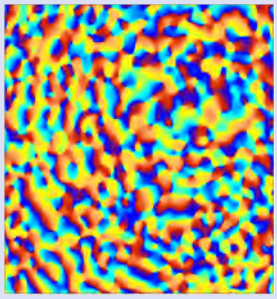


b) Intensity pattern of laser speckle transmitted through a strongly scattering sample.True size 10 ×10 *μm.*

[13] V. Ntziachristos, "Going deeper than microscopy: the optical imaging frontier in biology," *Nature Methods*, **7**, 603-614, 2010.

is stable. The average size of a speckle spot is determined by the geometry of the experiment. The Van Cittert-Zernike theorem shows that it is similar to the size of the focus of an ideal lens of the same size and distance as the random medium. The total number of spots is approximately equal to the number of degrees of freedom (modes) of the wavefront.

The phase of the light is coherent over an area comparable to a typical speckle spot as shown in panel (c), hence the area of a speckle spot is often indicated as a mode or a coherence area. Many subtle correlations in speckle patterns have been exploited for imaging.



c) Impression of the phase pattern corresponding to (b).

To a certain extent these effects amplify each other and progress in countering distortions (e.g. by adaptive optics) may lead to an increase in imaging depth as well as resolution. Importantly, recent progress on the above challenges has expanded our potential to peek into the structure and activity of biological matter:

a) Super-resolution microscopy, using stimulated-emission depletion or single-molecule localization microscopy, resolves living structures with <50 nm resolution, including nanometer-sized subcellular organelles and structures once thought to be inaccessible to visualization.
b) Multiconjugate adaptive optics[14] enhances contrast and resolution by compensating aberrations in the tissue.
c) Scattering has been tackled by a number of recent developments (see **Box 2**)
    i. Wavefront shaping methods use scattered light to focus on objects through strongly scattering layers.
    ii. Specially structured illumination such as Bessel beams may allow for deeper imaging through scattering media.
    iii. Acousto-optic wavefront shaping methods allow for optical imaging deep inside strongly scattering tissue, with a resolution that can be far superior to the typical ultrasound resolution.
    iv. Long-wavelength multiphoton (especially 3-photon) methods allow for high-resolution imaging at an increased depth up to 1 mm in scattering tissue as the long optical wavelengths are scattered less. However they are more sensitive to absorption.
    v. New developments allow for ever improving results of photoacoustic tomography, optical coherence tomography (OCT) and fluorescence diffuse optical tomography (f)DOT.
d) Multimode fiber endoscopes allow for minimally-invasive optical probing at any depth, through a fiber of outer diameter as small as 0.1 mm. As the image is guided in an optical fiber, absorption and scattering losses only occur in the small distance between the fiber tip and the object under study, allowing imaging at virtually unlimited depth. Sub-micron resolution has been demonstrated recently through such fibers[15].

It is important to note that most of the recent progress in this field has been aimed at medical applications, where the aim is to detect lesions, either in vivo or in histopathology, that support a diagnosis. This leads to quite different requirements from the ones for multiscale research into structure and function of the living brain. To allow for true multiscale research on a functioning mammalian brain, these emerging methods need to be combined and adapted to the specific challenges that brain research poses. In addition, completely new approaches are needed to fully address the challenge of deep imaging of brain functioning.

[14] M. J. Booth, "Adaptive optical microscopy: the ongoing quest for a perfect image," *Light: Science & Appl.* 3, e165 (2014).
[15] L.V. Amitonova, A. Descloux, J. Petschulat, M.H. Frosz, G. Ahmed, F. Babic, X. Jiang, A.P. Mosk, Ph. St. J. Russell, P.W.H. Pinkse, "High-resolution wavefront shaping with a photonic crystal fiber for multimode fiber imaging", *Opt. Lett.*, in press (2016).

Box 2: Shaping waves for imaging and focusing

Spatial wavefront shaping is a technique to control light in disordered media by manipulating the spatial phase of the incoming beam using a spatial light modulator (SLM). In its simplest version, the light at one particular point in or behind the medium is monitored, and an optimization algorithm is applied to maximize the intensity at that point.[16]

This procedure can increase the light intensity at the desired point by orders of magnitude, a factor that depends on the number of controllable pixels in the SLM. While still a small fraction of the incident light power, this optimized point can serve as an effective focal point and the basis of several imaging schemes, in particular, when combined with the 'memory effect,' that enables scanning this focus, albeit with a limited spatial range.

SCIENTIFIC VALUE AND EXPECTED BREAKTHROUGHS

ANTICIPATED PHOTONICS BREAKTHROUGHS

As outlined above, the aim of the NeuroPhotonics research facility will be to achieve breakthroughs in neuroscience through breakthroughs in biophotonics. Ultimately, the facility will feature technologies that enable neuroscientist to close the gap between studies on individual cultured neurons and studies using fMRI (**Figure 2**). We will now describe the anticipated technologies in more detail.

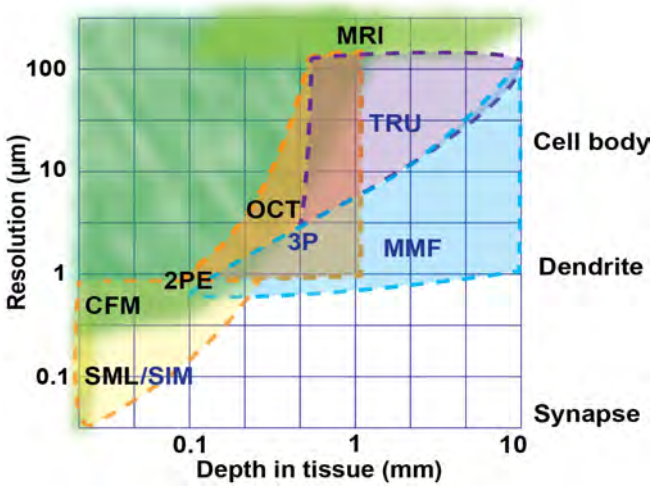


Figure 2: *Chart of resolution (in idealized conditions) versus depth for some existing and projected (dashed lines) optical methods in typical brain tissue.*
OCT= Optical Coherence Tomography
CFM= Confocal Fluorescence Microscopy
2PE= 2 photon excitation,
SML-= Single Molecule Localization methods,
3P = 3-photon excitation
MMF= minimally invasive imaging using multimode fiber probes
TRU= Time-reverse optics with ultrasound guide stars.
MMF and TRU methods hold the promise to specifically address neurons throughout the intact mouse cortex.

[16] I. M. Vellekoop and A. P. Mosk, "Focusing coherent light through opaque strongly scattering media," *Opt. Lett.* **32**, 2309-2311 (2007).

1. Optical nanoscopy on single cells in tissue

Recent advances in single-molecule localization microscopy as well as stimulated emission-depletion microscopy allow for systematic study of living cells at a resolution < 50 nm, in order to elucidate structural organization and subcellular dynamics in the neuron (**Figure 3**). Nevertheless, applying these techniques in complex tissue remains challenging and the use of adaptive optics is essential to allow superresolution imaging throughout the structure. In addition, the resolution and speed of current methods can be improved using an array of optical methods including intensity-, phase- and polarization-structured illumination.

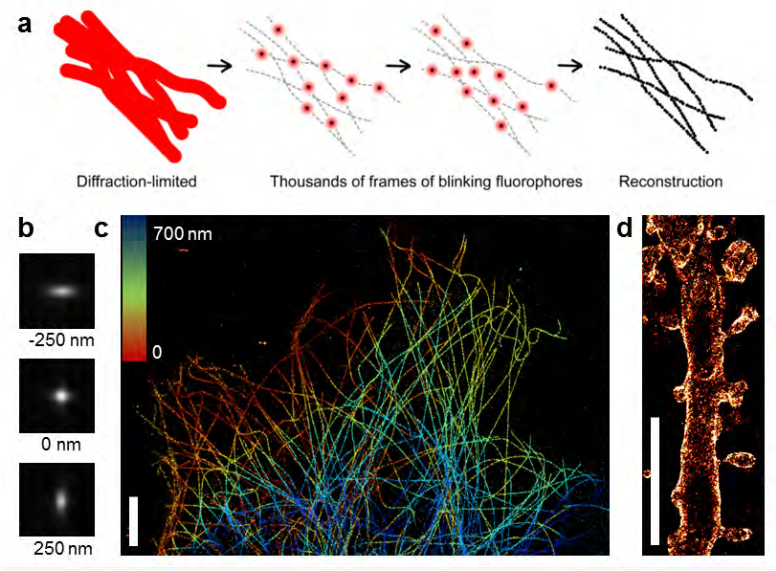


Figure 3: *Single-molecule localization microscopy (SMLM)*
a) achieving super-resolution by repetitive sparse sampling of individual marker molecules, followed by center analysis.
b) wavefront shaping can be used to correct aberrations and encode z-position.
c) 3D SMLM image of sub-cellular structures, made using adaptive optics.
d) Membrane imaging in live dissociated neurons.
Scale bar: 5 µm

2. Video rate sub-wavelength resolution structured illumination microscopy on few cells in tissue

To study the interaction between neurons in detail in 3D slices of tissue, a combination of high time resolution and high space resolution is needed. Structured illumination microscopy[17] uses correlations between projected illumination patterns (which may be computer-optimized or generated by analog gratings) and detected light. It allows for rapid volumetric imaging with a very good resolution approaching 100 nm, and its main advantage over molecular superresolution methods is its ability to rapidly image in a large field of view, making it an ideal method to study interacting neurons in 3D slices of tissue. In-tissue use of this method will require its combination with multi-conjugate adaptive optics to counter aberrations generated by several layers of cells.

3. Micrometer-resolution video-rate imaging in the cortex (<1.5 mm depth)

So far, it has not been possible to optically study the role of individual neurons in brain processes deep inside the cortex that involve hundreds or thousands of neurons. Multiphoton imaging methods, combined with advanced biochemical labelling and adaptive optics, may open a window into this intriguing regime. The crucial advantage of three-photon imaging is that fluorescent markers are excited by the simultaneous arrival of three long-wavelength photons. Due to the long wavelength of typically 1.7 μm, the influence of small scattering centers is suppressed and larger imaging depths are possible. These long wavelengths undergo slight absorption in tissue. This absorption limits the ultimate theoretical imaging depth to about 1.5 mm, while depths up to 0.9 mm have recently already been

[17] M. G. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," J. Microscopy **198**, 82-87 (2000)

demonstrated in living tissue [18]. The absorption also has a beneficial effect in that it suppresses scattered light. Combined with adaptive optics and advanced detection and data processing one may expect to reach sub-micrometer resolution at depths over 1 mm. This means that sub-cellular resolution can be obtained throughout most of a cortical layer. Specialized data-processing hardware and software will need to be developed to optimally use the data flow and forms an integral part of the NeuroPhotonics facility.

4. Cellular resolution at larger depths

When studying connectivity in even larger volumes of tissue, cellular resolution is often sufficient, while high data acquisition speed is crucial. The emerging method of time-reversed ultrasound encoded optics[19] offers a method to image with optical contrast, yet using acoustic waves which undergo almost no scattering in soft tissue (**Figure 4**). In this revolutionary class of methods, acoustic waves are launched into tissue to provide a "guidestar", after which wavefront shaping methods are used to launch specially shaped light waves that converge on the guidestar through the scattering medium[20]. It has been shown recently (in vitro experiments) that a resolution that is far better than the size of the acoustic focus can be reached. It is to be expected this method can be developed to yield sufficient resolution to address individual neurons through at least 3 mm of cortex.

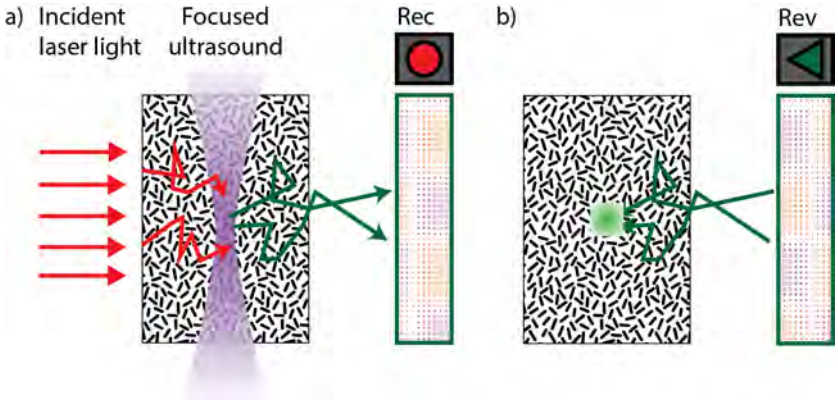


Figure 4: *Principle of time-reversed ultrasound-enabled (TRU) optical focusing. a) Incident laser light (red arrows) in a scattering medium is frequency shifted if on its diffuse path it passes through the focus of an ultrasound pulse. The frequency-shifted light is recorded holographically. (b) When the hologram is played back, the light makes it way back to the position of the ultrasound pulse. By scanning the ultrasound focus one can perform optical imaging at the resolution of the much less scattered ultrasound waves.*

5. Minimally-invasive (fiber endoscope) imaging through thin multimode fibers.

New fiber optic technology opens up the possibility to study processes inside single neurons at virtually any depth in the brain[21], while only minimally perturbing structure and function of the tissue covering the region of interest. This is accomplished by using thin multimode fibers as image guides. Such fibers, which have a thickness of only a few 100 μm, can be inserted into tissue without causing unacceptable damage. They are multimode waveguides, which means they guide complex fields such as images without appreciable loss. However, modal dispersion has so far precluded their use for imaging, as the image information is scrambled across thousands of waveguide modes with an a priori unpredictable random phase for each mode. Wavefront shaping has been shown to be a powerful method to undo the random phase shifts and hence to restore the image, either computationally or directly optically. The resolution can be subwavelength. Apart from forming images, the multimode fiber can also be used for spatially selective optical manipulation or activation.

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[18] N. G. Horton *et al.* "In vivo three-photon microscopy of subcortical structures within an intact mouse brain." *Nature Phot.* 7, 205 (2013)
[19] X. Xu, H. Liu, and L. Wang, *Nature Phot.* 5, 154 (2011)
20 R. Horstmeyer, H. Ruan, and C. Yang. "Guidestar-assisted wavefront-shaping methods for focusing light into biological tissue." *Nature Phot.* 9, 563 (2015);
[21] T. Cizmar, "Exploiting multimode waveguides for in vivo imaging", SPIE Newsroom DOI:10.1117/2.1201509.006106 (2015)

6. Transformative new photonic methods

The challenge of extracting functional (sub)cellular level information from networks in a living brain requires more than the extrapolation of methods envisioned today. Just as the development of new approaches and methods in photonics will enable new breakthroughs in neuroscience, the interdisciplinary approach including biology, physics, mathematics and computer science will promote breakthroughs in optics that today can only be seen "through a glass darkly". In a recent successful Lorentz Center workshop several disruptive new imaging paradigms were discussed, including compressive imaging[22], transmission matrix methods[23], structured illumination methods, lensless imaging and methods based on optical correlations. The impact of such methods on brain imaging is likely to be significant and active research into new paradigms will be conducted as part of the mission of NeuroPhotonics.

ANTICIPATED NEUROSCIENCE BREAKTHROUGHS

Closing 'the gap' in the study of brain tissue with non-invasive imaging methods provides many advancements of paramount importance in neurosciences. Not only does it enable imaging specific brain regions but, importantly, the proposed technical advancements would open up entirely novel domains of application.

- As the primary function of neurons is to electrically integrate dozens of distributed inputs simultaneously, 3D video rate imaging would make it tractable to get direct insights into neuronal information processing. Instead of being limited to imaging just single subdomains of a cell, it will become possible to study how multiple subcellular regions within a neuron produces computations. In addition, high resolution imaging across large fields of view would finally enable the long anticipated integrated study of neurons and glia cells, whose many regulatory functions are poorly understood. Furthermore, the different types of astrocytes and their extensive, but small-diameter extensions (< 200 nm) that penetrate many areas across large volumes of tissue, will finally also get into the picture. Furthermore, manipulating cellular domains with subcellular optogenetic tools will teach us how the morphodynamics of dendritic spines, the small micrometer-sized dendritic protrusions that harbor most excitatory synapses, contribute to learning and memory.

- The possibility to look deeper into the brain and extend the imaged brain volume beyond 1 mm from the surface **crosses a critical technical barrier in experimental neurosciences**. Imaging deep will make it tractable to visualize neurons at cellular resolution within key brain areas beyond the cortex. As such, functional read outs of brain can be extended to the substantia nigra (reward, addiction), amygdala (anxiety and emotions), thalamus (sleep and wake rhythms, epilepsy) and the hippocampus (learning and memory, Alzheimer, epilepsy). Furthermore, it allows the direct investigation how these brain areas **functionally interact via their longe-range circuit connections**. The mentioned brain regions have crucial roles in health and disease, including epilepsy, Parkinson and Alzheimer, but are presently outside of the imaging range of typical two-photon imaging techniques.

- While the mentioned applications above are important in fundamental neurosciences the innovative photonic imaging approaches will have major advantages beyond this and are promising for applied clinical research as well. For example, for imaging human pluripotent stem cells live during integration the ideal imaging technique would be sensitive enough to track individual cells as well as specific and safe enough for imaging in patients. With the proposed methods **non-invasive human stem cell tracking in larger brain volumes at cellular resolution** comes into reach and can be

[22] E. J. Candès, J.K. Romberg, and T. Tao, "Stable signal recovery from incomplete and inaccurate measurements" *Comm. Pure Appl. Math* **59** (8): 1207–1223. (2006);
D. L. Donoho, "Compressed sensing," *IEEE Trans. Inf. Theory* 52 (4): 1289–1306. (2006).
[23] S. Popoff, G. Lerosey, R. Carminati, M. Fink, A. Boccara, and S. Gigan, "Measuring the transmission matrix in optics: An approach to the study and control of light propagation in disordered media," *Phys. Rev. Lett*, 104, 100601, 2010.

adapted to image traumas more precisely, to guide brain surgery, or to trace in real-time cerebral engraftments at cellular resolution. Such methodology would be major step forward when compared to current tracking strategies based on MRI[24]. Importantly, the technical developments in photonics outlined above, not only have implications for neurosciences, but for life sciences in general and could eventually lead to new instruments for medicine or enable high-resolution studying of brain organoids.

SCIENTIFIC ADVANTAGES

Establishing the NeuroPhotonics Institute has many advantages for the Dutch science community and for the fields of photonics and neuroscience.

- First of all, it will provide Dutch scientists with an **internationally competitive research facility** to address key questions about brain functions.
- Combining the facility with fundamental research groups in photonics and neuroscience will ensure the **direct cross fertilization between photonics and neuroscience**. Because novel light sources, optics, detectors and data analysis methods are being developed in close collaboration with, and close proximity of, neuroscientists with relevant samples, new developments can be directly benchmarked and applied.
- Combining a facility with fundamental research groups in photonics and neuroscience will be the best way to **force breakthrough advances in photonics** aimed at **solving the most urgent questions in neurosciences in health and disease**.
- Finally, in the focused environment of the facility there will be a continuous exchange of ideas between the different disciplines. Photonic technology, developed by phyiscs and engineering groups, will enable breakthroughs in neuroscience, while conversely the complex challenges in neuroscience will stimulate development of optical and mathematical methods to deal with this complexity, **stimulating both neurosciences and biophotonics** within the facility and **within the Netherlands** as a whole.

RELATION OF THE FACILITY TO ALTERNATIVE FACILITIES AND METHODS

At the moment, there is no institute in the Netherlands that specializes in neurophotonics, nor a general facility for tissue-based imaging. The proposed KNAW large research facility for **Bioscopy** has the ambition to probe the properties of individual, isolated cells with atomic resolution by combining electron and light microscopy, nuclear magnetic resonance and proteomics. As such, it will complement our ambition to probe live cellular functioning within intact tissue. Similarly, the proposed KNAW research facility for **Neurotechniques** (Battaglia et al.) will be focused on brain-machine interfacing and seeks to integrate electrophysiology with robotics.

With respect to our ambitions, several Dutch research consortia and institutes are currently exploring novel imaging approaches and would be able to contribute expertise. Among the most relevant consortia and institutes are:

- The FOM-institute AMOLF in Amsterdam with several nanophotonics and biophysics groups
- LaserLaB Amsterdam, a collaboration of more than 10 laser- and biophysics research teams at the VU and UvA universities and the AMC and VUMC hospitals
- The ARCnl institute in Amsterdam, centered on the science of EUV light and novel imaging concepts
- The imaging physics center in Delft, which develop new imaging concepts

[24] Hunsberger JG, Rao M, Kurtzberg J, Bulte JWM, Atala A, LaFerla FM, Greely HT, Sawa A, Gandy S, Schneider LS, Doraiswamy PM (2015) Accelerating stem cell trials for Alzheimer's disease. *Lancet Neurol*.

- The Delft Centre for Systems and Control, which develops adaptive optics systems and concepts
- The Applied Nanophotonics and Biomedical Photonic Imaging cluster at the University of Twente
- The Optics Program and Van't Hoff Program at the applied research organization TNO develop new (bio)photonic technology

Importantly, we strive to establish an integrated infrastructure that combines photonics and neuroscience, which is currently non-existent in the Netherlands.

Optical methods to investigate tissue offer unique insights at scales from single organelles to cortex size, illuminating the areas where essential brain functions such as learning and memorization take place. In contrast to **electron microscopy**, which has a much higher resolution, it can probe intact and living tissue. The insights obtained through neurophotonics will supplement the vast knowledge gained from other modalities including imaging by fMRI, PET, CT and magnetoencephalography. Importantly, none of these alternative non-invasive methods are likely to reach the required resolution to probe many single neurons throughout the volume of brain.

-Magnetic resonance imaging allows for structural and functional imaging down to a scale of 0.1 mm. The minimal resolution is set by the strength of the field gradients needed for spatial selectivity as well as signal to noise considerations, as very small resolution voxels contain only a small number of oscillating dipoles. It is very unlikely that these limitations to the resolution can be overcome sufficiently to resolve single neurons.

-Positron emission tomography uses activated tracer molecules that emit positrons which diffuse over a small length before they recombine with an electron, releasing two coincident gamma rays that are detected by a detector array. The resolution is limited by detector physics and, more fundamentally, by the fact that the positron diffuses over a distance of order 100 μm before recombining.[25] In the light of this fundamental limitation to the resolution, this method cannot resolve single neurons.

-X-ray computed tomography (CT) imaging allows for structural imaging at a potentially very high resolution, however no functional information can be learned and the contrast in soft tissue is problematic. It is possible to use structural information from CT scans to enhance the resolution of optical methods.

-Magnetoencephalography measures the electrical activity of the brain through the weak magnetic fields generated by the impulses. Sensitivity and spatial resolution are very limited, making it impossible to pick up signals from a single neuron.

[25]W. W. Moses, Nucl. Instrum. *Methods Phys. Res*. A. 648 Supp. 1: S236–S240 (2011)

TECHNICAL CASE

TECHNICAL DESCRIPTION OF THE FACILITY

Above we have argued that addressing the big questions in neurosciences requires imaging the intact brain at high resolution and the controlled delivery of light deep inside tissue. These challenges cannot be addressed by the neuroscience discipline alone. Fortunately, several developments in (bio)photonics and the existing excellent groups in biophotonics in the Netherlands are offering potentially innovative imaging techniques that could enable this ambitious goal. We propose to combine and merge excellent groups from these disciplines within a single research facility providing Dutch neuroscience and biophotonics community a novel platform for world-class research, thus accelerating both neuroscience and photonics research. In this section, we will provide a technical description of the institute in terms of structure and operation. The institute will host a core facility of state-of-the-art instrumentation for optical stimulation and imaging that will be accessible for external users. However, unlike other high-end life science equipment, such as mass spectrometers, NMR devices and electron microscopes, most of the technology that will be provided by our facility is not commercially available as complete instruments. Instead, it either still needs to be developed or is currently only available in highly specialized research groups. Given the extreme modularity and customizability of optical setups and the highly specialized experiments for which they are used, we anticipate that integrated turn-key instruments that cover the demands outlined above will not become available. Instead, such tailored instruments need to be developed in and maintained by specialized photonics groups. Therefore, **a neurophotonics facility cannot exist without dedicated research groups that will continue to push the boundaries of technology development and application and ensure that the core facilities remain operational and state-of-the-art.** To allow rapid technological advances, the institute will also have various in-house support facilities, such as a mechanical workshop, electronics workshop, IT/data management support facility and an animal facility. The research groups will be supported by different facilities that enable effective instrument development and testing. In turn, the research groups are responsible for proper maintenance and improvement of the core facility for external users. To stimulate and accommodate facility access by external users, a dedicated facility office will be established, next to the general management. The structure of the institute is depicted in **Figure 6** and will be described in more detail below.

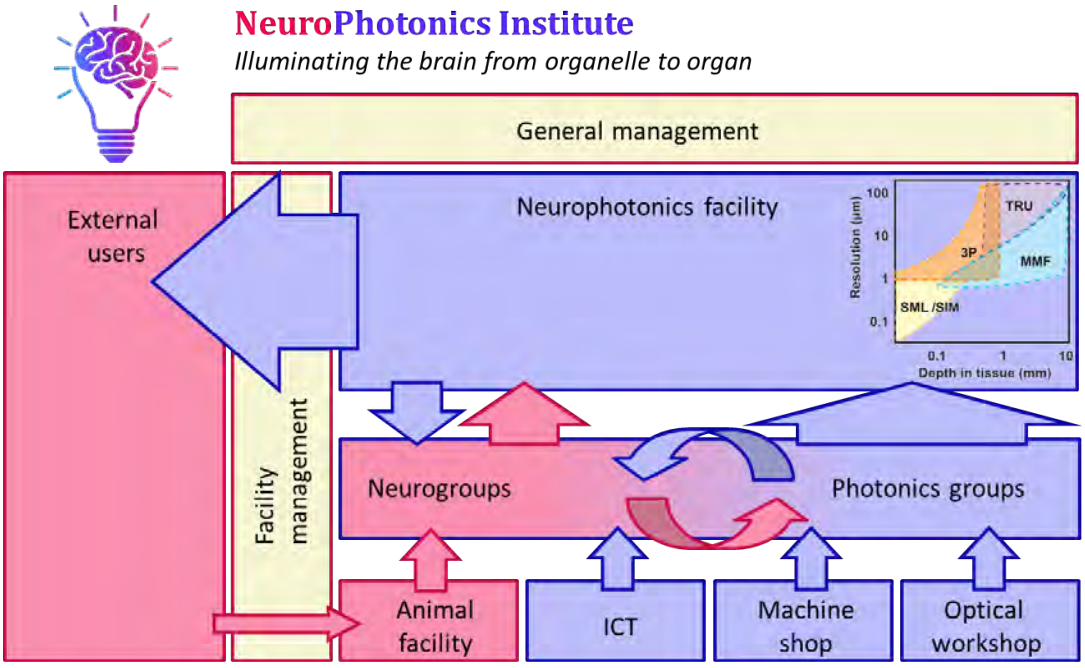


Figure 6: *proposed structure of the NeuroPhotonics Institute, designed to guarantee state-of-the-art equipment and to stimulate external access. Arrows depict the flow of technological know-how and equipment (blue) or biological know-how and specimens (red).*

- **Scientific groups:**

We expect that a multi-disciplinary team of ~10 scientific groups will be sufficient to establish a core of expertise required for proper functioning of the facility. Several neuro-oriented groups with a strong interest in novel methodology will establish and exploit relevant model systems, such as slice cultures and transgenic mouse lines, and develop and exploit wet methodology, such as molecular biology tools and protocols for fluorescence microscopy and optogenetics (vectors, sensors, stimulation protocols). The groups will be augmented by a variety of physics-oriented groups focused on superresolution microscopy, structured illumination, wavefront shaping, and fiber-based imaging. In addition, at least one group will heavily focus on challenges in data handling and analysis, in particular for image-based data. Together, these groups should be able to achieve the technological breakthroughs described in the scientific case, which will then facilitate the neuroscience breakthroughs by external (and internal) users.

Once technology is robust enough to allow external access, a streamlined version of the instrument will be placed in the general facility. Maintenance and upgrading of instruments in the general facility will stay under the responsibility of the scientific group of origin.

- **Support groups:**
  - Animal facility
  - Machine shop
  - Optical workshop: design, custom assemblies, product selection
  - ICT/Electrical engineering/Data management

The machine shop and optical workshop will both comprise 3 FTE and basic instrumentation. The staff will concentrate on advice, design and assembly, while most production activities will be outsourced to commercial parties. Similarly, the animal facility will be relatively lean and we will explore the possibilities to outsource breeding activities to nearby facilities. The ICT support will not just cover basic ICT needs and support, but also implement advanced data management and analysis infrastructure, as well as aid in computer-based control of equipment.

- **NeuroPhotonics facility:**

The exact array of instruments in the facility will change continuously, but should in the end cover the entire range of depth and resolution outlined earlier. In the initial stage, the following equipment will be available:

  - Laser park, including advanced pulse-shaped infrared femtosecond and picosecond sources.
  - Multiphoton microscopes
  - Super-resolution microscopes
  - Structured-illumination microscopes (including light-sheet microscopy)
  - Endoscopic instrumentation
  - Tissue preparation equipment
  - In vivo recording setups
  - Equipment for 3-photon imaging and multimode fiber endoscopy.
  - Research setups for acousto-optic wavefront shaping and time-reversed ultrasound optics.

- **Facility management:**

External users can apply for access to instrumentation using a brief application form that describes the scientific case and the equipment of choice. An internal committee will judge these applications, based on feasibility and novelty. The facility management will be the central point of contact and assistance for all users, both during the application and the actual experimental stages. They will assist in experiment scheduling, animal arrangements, travel arrangements, data handling etc. We expect that the facility management will comprise 3 FTE, including administrative handling and hosting activities.

- **General management:**

Finally, the institute requires a general management that comprises a managing director, administrative staff and finance and control. This will also comprise 3 FTE.

BALANCE BETWEEN EXISTING TECHNIQUES AND INNOVATION

We propose to establish technology to image the brain at greater depth and at higher resolutions. The capabilities of existing techniques with respect to depth and resolution are depicted in **Figure 1**, whereas our goals are depicted in **Figure 2**. Closing the gap between these two figures requires the careful optimization and customization of existing techniques, but also demands more innovative approaches. Multiphoton microscopy, optical nanoscopy and several types of adaptive optics are already operational in the laboratories of the applicants and are becoming more and more mature technologies. Further optimization of these techniques will already progressively push the boundaries to reduce the white areas in **Figure 2**. Nevertheless, full closure will also require radical new technology, as argued earlier.

CHALLENGES AND RISKS

The general risk with facilities is that they are quickly outdated if the technology is not maintained up to date. This is especially a big concern in the rapidly changing landscape of neurophotonics. The proposed facility combines a core set of instruments accessible for external users, with a research core of dedicated groups that work on technology development and groups that pursue important questions about the brain and depend on novel technology. This structure will ensure continuous development and upgrading of the facility's core instrumentation.

The crucial, defining capability of the facility will be imaging on any size scale, from synapse to systems level. This requires the development of many methods that are now in the in vitro or even proof-of-concept stage, and to bring them into an operational stage of in-vivo optics. This is a huge and inherently risky step.

However the risk is mitigated by the availability of a wide range of different technologies (some shown in **Figure 2**) which have overlapping areas of operation. For some methods, such as 3-photon imaging, in vivo measurements were recently demonstrated[26]. Multimode fiber endoscopes are under intense development. Rigid fibers in a steel jacket will almost certainly work in vivo as well as in vitro, while more flexible solutions (which are very desirable) may require years of additional research.

A second risk inherent in fast volumetric imaging is that of data overload. In the most optimistic case, data can be collected from thousands of neurons with high time resolution. The amount of data gathered from a single mouse brain can be several gigabytes per second. Processing and filtering this data with methods and tools typical of life science experiments may be too slow by orders of magnitude. To counter this possible data overload, methods and tools from disciplines that are comfortable with even much larger data streams will be applied. Experts form the fields of earth observation and high-energy physics will be actively consulted, and experts on data processing will form part of the institute's team.

[26] Horton, Nicholas G., et al. "In vivo three-photon microscopy of subcortical structures within an intact mouse brain." *Nature photonics* 7.3 (2013): 205-209.

B. EMBEDDING

RELATED FACILITIES OUTSIDE THE NETHERLANDS

For quite some time, big facilities were the realm of astronomy and particle physics to build large telescopes or accelerators. More recently, big facilities are increasingly established for biology, such as synchotrons and free electron lasers for structures determination, as well as dedicated proteomics and bioinformatics facilities. In contrast, large national facilities for light-based imaging are still rare, because microscopy is so essential for day-to-day research that every department or institute has its own facility. Nevertheless, the last decade has witnessed tantalizing developments in light microscopy that require extremely advanced instrumentation that can no longer be set up and maintained by individual local facilities. As a consequence, hotspots for advanced imaging are now emerging in Europe and the US where new technology is developed and made available via core facilities. The most important examples are:

- Janelia Research Campus, Ashburn, VA, USA (https://www.janelia.org/)
- European Molecular Biology Laboratory, Heidelberg, Germany (http://www.embl.de/)
- Max Planck Institute for Biophysical Chemistry, Göttingen, Germany (http://www.mpibpc.mpg.de/)
- Interdisciplinary Institute for Neurosciences, Bordeaux, France (http://www.iins.u-bordeaux.fr/)
- National Center for Microscopy and Imaging Research, San Diego, CA, USA (https://ncmir.ucsd.edu/)

The proposed facility will be unique in the Netherlands and will integrate and facilitate research activities that are now scattered over different universities, resulting in limited integration between development and applications. In some ways, the proposed facility has a structure similar to HHMI Janelia Research Campus, which features both fundamental research groups and groups that heavily focus on technology development and release these into facilities open for external users. Relevant examples of such facilities are the iPALM setup for 3D localization microscopy developed by Harold Hess and the lattice light sheet microscope developed by Eric Betzig.

Within Europe, the EMBL in Heidelberg features a well-equipped advanced imaging center that can be accessed upon request. However, this institute is not focused on neuroscience and does not feature the dedicated instruments required to study the brain. In 2011, the Interdisciplinary Institute for Neuroscience was established in Bordeaux, France (http://www.iins.u-bordeaux.fr/). As can be read on their website, the aims of the IINS are to understand the basic mechanism in the nervous system through development of new (optical) methods that will be made available to the community through the core facility and training activities.

CONNECTION WITH STRONG DUTCH RESEARCH THEMES

The Netherlands has a long-standing tradition in advanced microscopy and photonics. Its heritage includes many pioneering developments, starting from the work of van Leeuwenhoek in the 17th century, with more recent milestones being the invention of phase contrast microscopy (Nobel Prize, Zernike), the development of episcopic illumination (Ploem) and the development of confocal microscopy as a tool for biology (Brakenhoff). Today, photonics is a cornerstone of academic research as well as high-tech industry (e.g. ASML, Philips). Among current developments with international impact in biophotonics are wavefront shaping methods to image through opaque layers (Twente), single-fluorophore optical methods (Leiden, VU, Utrecht, Delft), acousto-optic methods (Twente, Rotterdam, Philips), instrumentation for tissue optics (Eindhoven, Twente, Groningen, Maastricht, Delft, VU, TNO, Philips) and advanced adaptive optics microscopy (Delft, Leiden, Utrecht).

The Netherlands has also a vivid neuroscience community, which on the one hand features a great strength in cellular and systems neurosciences utilizing *in vitro* or *vivo* recordings (e.g. VU, KNAW Netherlands Institute for Neuroscience, Erasmus MC, Donders Institute in Nijmegen) and on the other

hand a strong expertise in functional brain imaging using MRI and PET (e.g. the UMC Utrecht Hersencentrum, the Spinoza center for Neuroimaging in Amsterdam, the Max Planck Institute for Psycholinguistics in Nijmegen, and the Neuroimaging Center in Groningen). In addition, research into neurodegenerative and neuropsychiatric diseases is highly developed (UMC Utrecht, RU Groningen). Importantly, the proposed facility will provide the required infrastructure to connect the distinct time and length scales of cellular recordings and MRI/PET-based imaging and to open the window into the brain regions where degenerative diseases start. Developing the NeuroPhotonics Institute will provide various user groups in the Netherlands with an internationally competitive research infrastructure. Performing research in a multidisciplinary organization will promote the development of novel technologies, foster mutual interactions and new types of collaborations. This is expected to lead to a significant increase in the research excellence. Furthermore, the strong rise in neuroscience Master programs across Dutch universities and the increasing numbers of (inter)national students indicates a major potential for research and training activities within the Netherlands. Furthermore, interactions with the excellent KNAW Hubrecht Institute, investigating human stem cell integration in the brain and brain organoids are also foreseen.

ECONOMIC AND SOCIETAL ADVANTAGES

Scientific breakthroughs occur not only by research excellence within a single discipline but also through multidisciplinary research combinations, creating new opportunities in the research landscape. Indeed, increased collaboration between scientific disciplines and industry partners has been recognized as important factors to ensure impact on Dutch sciences, society and economy[27]. The NeuroPhotonics institute will remove barriers between two major disciplines; neurosciences and physics. While historically there have been many important connections between both disciplines, in particular through electrical engineering, to establish novel light-based technologies more cross-discipline interactions are required. As such, the institute will contribute to cutting-edge scientific research output, research valorization and education. Training of multidisciplinary MSc and PhD students will be an important task of the facility, to maintain a competitive national educational level compared with other European countries, where cross-disciplinary institutes are currently already developing or established. Finally, economical advantages can be expected from the establishment of spin-off companies that provide valorization of the cutting-edge techniques developed in the institute (discussed in C and D).

The NeuroPhotonics Institute will be of major societal relevance. The government recently assigned *Brain, Cognition and Behavior* as one of the sixteen core themes of the Dutch science landscape based on the outcomes of the 'Nationale Wetenschapsagenda'[28]. In fact, the most frequent question submitted concerned the fundamental mechanisms and potential treatments of brain diseases, in particular Alzheimer's disease[29]. In addition, the impact of brain diseases, including psychiatric and other neurological disorders, on the national health care costs is about one-third of the national budget (~10 B€)[30]. Through partnerships with research organizations such as the KNAW Hubrecht Institute, the KNAW Netherlands Institute for Neurosciences and the university departments we believe that the NeuroPhotonics research facility will directly facilitate the study of brain diseases as the non-invasive deep and high-temporal resolution imaging will enable the optical access to critical nodes in brain disease.

[27] Wetenschappelijke Raad voor het Regeringsbeleid (2013), Naar een lerende economie, Amsterdam University Press, Amsterdam

[28] Kabinetsreactie_Nationale_Wetenschapsagenda.pdf, 27 november 2015

[29] Nationale wetenschapsagenda (http://www.wetenschapsagenda.nl/)

[30] https://www.hersenstichting.nl/

C. ORGANIZATION AND FINANCING

ORGANISATION

SCIENTIFIC EXPERTISE

Successful establishment and operation of the NeuroPhotonics Institute requires expertise from both neuroscience and photonics. The required mix of expertise is nicely reflected in the backgrounds and respective fields of the applicants:

- **Maarten Kole** studied Biology at the university of Groningen. He performed his PhD in electrophysiology at the Leibniz Institute for Primate Research (Göttingen, Germany, 2003) and his postdoctoral work at the ANU (Australia). Here, he achieved the first direct state-of-the-art recordings of action potentials in small diameter mammalian axons providing the unequivocal description of the mechanistic origin of neuronal spikes. In 2011 he became a group leader at the Netherlands Institute for Neuroscience, Amsterdam and in 2014 was appointed professor on the special chair Biophysics of complex cellular systems, founded by the Stichting Physica at Utrecht University. Maarten Kole is recipient of AW Campbell Award (2010) and an ERC Starting Grant (2011). He currently focuses on the continuing development and application of novel biophysical methods and recording tools. In his research group high-resolution VSD imaging methods of submicron domains in axons are established. He is committed to link fundamental biophysical insights in neurons and glia to a new understanding of demyelinating diseases. Key publications:
  - Hallermann S, de Kock CP, Stuart GJ, Kole MHP. *State and location dependence of action potential metabolic cost in cortical pyramidal neurons.* Nat Neurosci. 15, 1007-1014 (2012).
  - Kole MHP, Stuart GJ. *Signal processing in the axon initial segment.* Neuron. 73, 235-247 (2012).
  - Battefeld A, Klooster J, Kole MHP. *Myelinating satellite oligodendrocytes are integrated in a glial syncytium constraining neuronal high-frequency activity*. Nat Comm. 7, 11298 (2016)

- **Allard Mosk** studied Physics and obtained his PhD from the University of Amsterdam in 1999, after which he performed post-doctoral research at the Max-Planck Institute for Nuclear Physics in Heidelberg, the Ecole Normale Supérieure in Paris and the FOM-institute for plasma physics in Rijnhuizen. He predicted and observed novel phenomena in ultracold atoms, including light-induced formation of hydrogen molecules and the possibility of negative Kelvin temperatures in gases. After he moved to the field of light in complex systems, he opened the field of wavefront shaping in scattering media and is now widely recognized as an innovator in the field of optics in complex media[31]. In 2015 he was part of a DARPA-funded science team that examined the prospects for wavefront-shaping and related methods for deep tissue probing, finding that these methods are excellently suited for transformative applications in neuroscience, which he is eager to develop in an interdisciplinary setting. He recently became chair of the new Physics of Light in Complex Systems group at Utrecht University (www.nanolinx.nl). He is a fellow of the OSA and recipient of Marie-Curie, ERC consolidator, NWO-Vidi and NWO-Vici grants. Key publications:
  - Vellekoop IM, and Mosk AP, *Focusing coherent light through opaque strongly scattering media*, Opt. Lett. 32, 2309-2311 (2007).
  - Bertolotti J, Van Putten EG, Blum C, Lagendijk A, Vos WL, and A. P. Mosk , *Non-invasive imaging through opaque scattering layers*, Nature 491, 232-234 (2012).
  - Yilmaz H, Van Putten EG, Bertolotti J, Lagendijk A, Vos WL, and Mosk AP, *Speckle correlation resolution enhancement of wide-field fluorescence imaging,* Optica **2**, 424-429 (2015).

[31] Merali Z (2015) Optics: Super vision. *Nature* 518:158-160

- **Lukas Kapitein** studied Physics at the VU University in Amsterdam, where he also obtained his PhD in 2007 in Biophysics for single-molecule studies on molecular motors. During his postdoc in Neuroscience at the Erasmus Medical Center, he used live-cell microscopy and molecular engineering to examine how intracellular transport by molecular motors underlies neuronal architecture and functioning. In 2011, he established his own research groups at Utrecht University, which is aimed to develop and apply light-based technology, such as super-resolution microscopy and optogenetics, to dissect and direct the cellular processes that drive neuronal development and functioning. He is a recipient of Veni, Vidi and ERC grants. Two recent breakthrough methodologies from his group now allow 1) controlling intracellular neuronal transport using light [32] and 2) resolving bundled microtubules (tracks for motor proteins) in cultured neurons. His next ambitions are to apply these methods within intact brain tissue. Key publications:
  - Mikhaylova M, Cloin BM, Finan K, van den Berg R, Teeuw J, Kijanka MM, Sokolowski M, Katrukha EA, Maidorn M, Opazo F, Moutel S, Vantard M, Perez F, van Bergen en Henegouwen PM, Hoogenraad CC, Ewers H, Kapitein LC. *Resolving bundled microtubules using anti-tubulin nanobodies*. Nature Communications 6:7933 (2015)
  - Van Bergeijk P, Adrian M, Hoogenraad CC, Kapitein LC. *Optogenetic control of organelle transport and positioning*. Nature 518:111-114 (2015)
  - Adrian M, Kusters R, Wierenga CJ, Storm C, Hoogenraad CC, Kapitein LC. *Barriers in the brain: resolving dendritic spine morphology and compartmentalization*. Frontiers in Neuroanatomy 4:142 (2014)
- **Marloes Groot** studied Physics at the VU University in Amsterdam, where she obtained her PhD in 1997 in Biophysics studying photosynthesis. She performed postdoctoral research at the University of Chicago where she was the first to perform color-resolved three-pulse photon echo spectroscopy, on bacterial reaction centers. After that she moved to the Ecole Normal Superieure de Techniques Avancees (ENSTA) where she built a setup and recorded coherent infrared emission from myoglobin crystals. Upon return to VU University she developed a versatile setup for femtosecond visible/midinfrared pump-probe spectroscopy to study protein dynamics. In 2010 she developed a setup based on third harmonic generation microscopy, to study live cell dynamics, label-free in deep-tissue, in collaboration with researchers from the Neuroscience Campus Amsterdam. Since 2012 she develops this technique further for the diagnostics of brain tumors, both *ex-vivo* and *in-situ* using microendoscopics. Key publications:
  - Witte S, Negrean A, Lodder JC, De Kock CPJ, Silva GT, Mansvelder HD, Groot ML*Label-free live brain imaging and targeted patching with third-harmonic generation microscopy* Proceedings of the National Academy of Sciences 108, 5970-5975 (2011)
  - Witte S, Plauşka A, Ridder MC, Van Berge L, Mansvelder HD, Groot ML, *Short-coherence off-axis holographic phase microscopy of live cell dynamics* Biomedical optics express 3, 2184-2189 (2012).
  - Kuzmin NV, Wesseling P, De Witt Hamer PC, Noske DP, Galgano GD, Mansvelder HD, Baayen JC, Groot ML, *Third harmonic generation imaging enables fast, label-free pathology of human brain tumors* submitted for publication
- **Corette Wierenga** studied Physics at the VU University in Amsterdam, followed by a PhD (2002) in neurobiology at the University of Amsterdam. During her PhD thesis, she studied the role of interneurons in the hippocampal CA1 area. She worked as a postdoctoral researcher at Brandeis University and at the Max Planck Institute of Neurobiology in Martinsried. In 2011, she started her own group at Utrecht University, where she is Associate Professor since 2015. Her main expertise is in the formation and plasticity of inhibitory synapses in dendrites of principal neurons, with a special emphasis on the local interactions with excitatory synapses. In her lab, she combines two-photon microscopy and electrophysiology in acute brain slices and organotypic cultures. She received

[32] Dance A (2015) Micromanagement with light. *Nature* 528:291-294

several grants, including the Marie Curie intra-European Fellowship, a Reintegration Grant, a ZONMW-VIDI and was awarded the Young Physiologist prize and L'Oreal award for her research. Key publications:

- Wierenga CJ, Becker N, Bonhoeffer T, *GABAergic synapses are formed without the involvement of dendritic protrusions*. Nature Neurosci, 11: 1044-1052 (2008)
- Schuemann A, Klawiter A, Bonhoeffer T, Wierenga CJ, *Structural plasticity of GABAergic axons is regulated by network activity and GABAA receptor activation*. Frontiers in Neural Circuits 7: 113. (2013)
- Müllner F, Wierenga CJ*, Bonhoeffer T*, *Precision of inhibition: Dendritic inhibition by individual GABAergic synapses on hippocampal pyramidal cells is confined in space and time*. Neuron 87, 576-589 (2015) * co-senior author

Together these groups encompass the core expertise required for guiding the initial ramp up phase of the infrastructure. Nevertheless, they have no current expertise in time-reversed ultrasound encoded optics and massively parallel data acquisition and processing. During the coming years, complementary teams that could strengthen the institute in these areas will be identified.

ORGANISATION STRUCTURE

The global structure of the mature, fully operational facility (~10 years from now) has been described in the technical case. In the coming years, the applicants will form the steering committee of the institute to organize the preparatory scientific and organizational activities required to establish the institute. The steps that will be taken are outlined in section *D: Further Developments*.

FINANCING AND PROPOSED BUSINESS PLAN

ESTIMATED COSTS OF REALIZATION

We expect the facility to be fully operational in one building as of 2024. From that point, PhD and postdocs will be covered from external funding sources. The 10 scientific groups (10x PI + technician) comprise 20 FTE. The support facility will require 12 FTE, the facility management 3 FTE and the general management 3 FTE. In total, the facility will have 38 FTE.

During the first years, upfront financial support will be used to stimulate scientific development and strengthen the collaborative projects within the multi-disciplinary consortium of the NeuroPhotonics. In this stage, several PhD students and postdocs will be appointed on collaborative projects and new instrumentation will be developed. During the first years, external expertise will be hired to establish legal policies and institute infrastructure, website design, and to organize plenary meetings with stakeholders and the representatives of the top-sectors.

Gradually, new groups will be attracted that require housing, support staff, equipment and running budget. For housing costs, we estimate 70 k€ per research group. This is a relatively standard estimate in institutes that operate in a so-called total-cost model. Although we currently think that a new building (as of 2024) will be the best way to realize the facility, other options (such as rental) could work as well. A new science building for 10 research groups, facility equipment and support staff will cost between 15-20 M€ to build and has a typical life time of 30 years. The effective costs for the period of 2024-2034 will therefore be 6-7 M€, which is 60-70 k€ per group per year. Running costs comprise all costs needed to be operational, including electricity, gas, IT services, cleaning and general supplies. These are estimated at 30 k€ per year per group during the period until 2024 and 100 k€ per year per group in the period from 2024-2034. Estimated cost per FTE is 75 k€.

We estimate the total investment in equipment to be around 20 M€, averaging to 2M€ invested per group. High-end commercial microscopes with STED and Single-molecule localization capabilities often cost between 0.5-1 M€, whereas a single IR femtosecond pulsed laser source, as needed for multiphoton microscopy, typically costs around 0.1 M€. The costs of most other optical components, such as CW laser

sources or Spatial Light Modulators are largely below 50 k€, but quickly add up to 250-500 k€ in a high-end custom imaging setup. On the whole, we expect that each group on average has 2 setups for technique development and 2 set ups that run within the facility. These 40 setups, costing 0.25 to 1 M€ each, will add up to 20 M€.

year	groups	FTE	staff	equipment	housing	running cost	annual costs
2016	2	-	-	100	-	60	160
2017	3	3	225	200	-	90	515
2018	4	4	300	200	280	120	900
2019	5	4	300	500	350	150	1,300
2020	6	6	450	800	420	180	1,850
2021	7	6	450	1,000	490	210	2,150
2022	8	8	600	1,500	560	240	2,900
2023	9	12	900	2,000	630	270	3,800
2024	10	16	1,200	3,000	700	1,000	5,900
2025	10	20	1,500	3,000	700	1,000	6,200
2026	10	30	2,250	2,000	700	1,000	5,950
2027	10	38	2,850	1,500	700	1,000	6,050
2028	10	38	2,850	1,200	700	1,000	5,750
2029	10	38	2,850	1,000	700	1,000	5,550
2030	10	38	2,850	700	700	1,000	5,250
2031	10	38	2,850	500	700	1,000	5,050
2032	10	38	2,850	300	700	1,000	4,850
2033	10	38	2,850	200	700	1,000	4,750
2034	10	38	2,850	200	700	1,000	4,750
Total (k€)			30,975	19,900	10,430	12,320	73,625

Budget table: *Expected costs of establishment and operation of the NeuroPhotonics Institute. The gray box marks the period during which the facility is located in one building. The number of groups in the starting periods reflects the sum of fractional activities of the founding groups (applicants plus newly affiliated groups), because these groups will initially also maintain research activities independent of the institute. Initial FTE count includes PhD students and postdocs, but later FTE counts only include scientific and support staff, because PhD students and postdocs will be externally funded.*

EXPLOITATION

It is currently envisioned that the the required annual ~5-6 M€ will be publicly funded and act as an investment with various types of returns. First, through this funding the institute will provide a major impetus to Dutch sciences, likely to lead to secondary effects such as patents in photonics, spin-off companies, employment of academic and non-academic personnel, including PhDs, postdocs and principal investigators. The cross-disciplinary interactions between photonics and neurosciences by the clustering of excellent research groups is likely to strengthen the chance for successful application for competitive EC funding, including the ERC, ERC PoC and H2020 grants, private parties and foundations as well as the acquisition of national grants such as NWO/FOM program grants or NWO (middle) Groot. While the success of the Netherlands is currently very high in most EC programs (Rathenau report), to maintain this competitiveness there is the need to invest in new research directions and prepare for changes in the international research landscape. Although there are some links between the Dutch neuroscientists and HHMI Janelia Research Campus, developing and applying photonic techniques locally in the Netherlands will provide a cutting-edge advantage that accelerates discoveries and increases our chance for maintaining a high position in the various science rankings. The multidisciplinary approach of the institute is in line with some developments abroad. In this respect, establishing a NeuroPhotonics Institute in the short term will have a timely cutting-edge advantage. It is a different strategy of performing science, based on the breadth of constituents with distinct backgrounds and viewpoints that focus a singular problem.

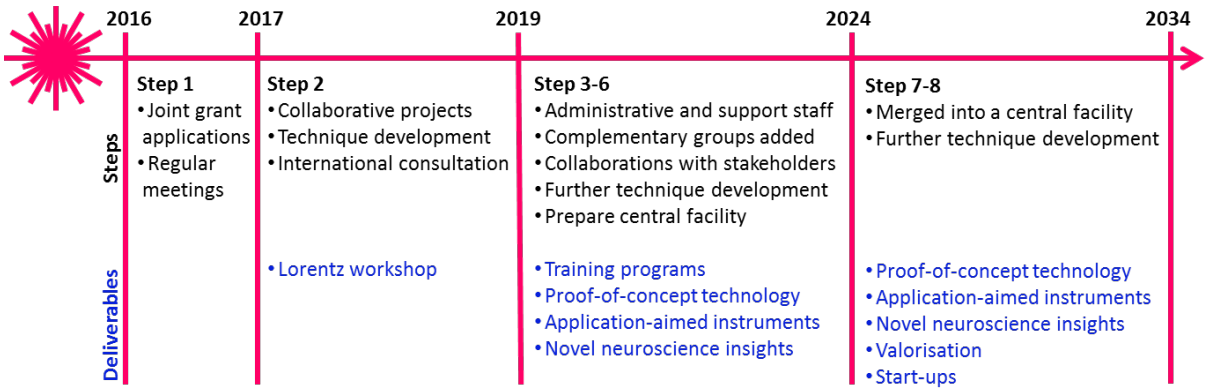
- Since the light-based innovative techniques for optically imaging and controlling brain dynamics will be readily available to national partners this may accelerate the quality and quantity of brain research in the Netherlands. Furthermore, the institute will act as an important platform for the academic training of highly skilled high-tech PhD students and postdocs leading to internationally increasing the research capacity and collaboration with other neurophotonics centers in fundamental as well as applied brain research.

- We anticipate that the NeuroPhotonics Institute will not only benefit national research platforms but also **attract investments from industry partners and generate economic value via spin-offs and join ventures.** The Netherlands is world leading in the design, development and production of high-tech equipment and micro- and macro-components. In fact, high-tech is the second largest component of the top sector [33]. At this moment there are about 150 Dutch industrial and scientific partners active in photonics with partners coming from the industry (Philips, ASML, TE Connectivity), small medium enterprises, TNO, Technical Universities of Eindhoven, Twente and Delft as well as institutes like AMOLF, ASTRON, SRON [34]. The added value of the high-tech top sector is estimated at B€23. For photonics alone it is estimated at B€2 per year (EC, 3rd European report on S&T indicators, 2003). The excellent position of Dutch universities is exemplified by covering almost 35% of the 60M€ European photonic projects on photonic integration technologies. In the recently published vision document 2016 – 2019 the HTSM top sector expressed the ambition to increase its export to M€ 75 in 2025 and the production to M€ 182. A significant increase in the public investment to research in the technology is thereby considered to be inevitable [35]. We expect about 3 spin-off companies that will attract entrepreneurs and investors.

D. FURTHER DEVELOPMENT

The applicants are currently forming the steering committee of the NeuroPhotonics Institute. To further develop the large-scale facility the following steps are needed.

1. Research activities of the different teams will become thematically aligned and focused on the goals described above
2. National and international excellent research groups in photonics and neuroscience will be consulted to further develop the scientific and technical case
3. Formal administrative and technical support will be initiated
4. Complementary groups will join the (by then still virtual) institute and receive financial support
5. Training programs will be developed
6. Collaborations with national stakeholders in private industry, universities and other relevant organizations in the Netherlands will be established to support the facility
7. The research activities will be concentrated in a state-of-the-art building
8. The NeuroPhotonics Institute is fully operational as a center of expertise and research facility.



[33] http://topsectoren.nl/high-tech
[34] Holland Photonics Roadmap, 29 November 2012. (www.rvo.nl/).
[35] High Tech Systemen en Materialen, Kennis en Innovatie Agenda 2016-2019.

STEP 1-2: INITIAL ORGANIZATION OF THE NEUROPHOTONICS INSTITUTE

In the early ramp up phase, the steering committee will perform the administrative tasks of the institute, develop the scientific collaborative projects and have regular meetings to establish further organizational structure. In addition, scientific advisers will be consulted to further develop the scientific and technical case and to identify complementary expertise that could join the NeuroPhotonics Institute. To achieve integration with European consortia, we will seek advisory roles from Prof. Dr. Daniel Choquet (Bordeaux, France) and Prof. Dr. Anthony Holtmaat (Univ. Geneva, Switzerland). Visibility of the institute will be promoted by establishing a website http://www.NeuroPhotonics.nl, where news and progress updates will be posted. Concrete steps that will be taken are:

- To develop new optical imaging strategies, it will be essential to foster scientific collaborative efforts between the members of the steering committee. Current discussions within the core group have led to well-developed plans for collaborative NWO and/or FOM program grants that will be submitted in 2016 to support the scientific alignment of research techniques.
- The steering committee will submit a proposal to the Lorentz Center for a one-week workshop on '*Neurophotonics, illuminating the brain from synapse to system*. The Lorentz Center (http://www.lorentzcenter.nl/aim.php) is an international center that coordinates and hosts workshops in the sciences, based on the philosophy that science thrives on interaction between creative researchers. Lorentz Center workshops focus on new collaborations and interactions between scientists from different countries and fields, and with varying seniority. One of the applicants, Lukas Kapitein, already organizes a workshop on Optogenetics in March 2016, which is aimed at identifying molecular design principles for effective optogenetic modules. The Neurophotonics workshop, projected in 2017, will discuss the most effective strategies for high-resolution, high-speed optical imaging deep into intact brain tissue. This will be an excellent opportunity to launch the (by then still virtual) NeuroPhotonics Institute and establish connections with international leaders in the field.
- The most promising methods and techniques for the early operational stage of the institute will be defined and applied physics research will be initiated to make them ready for application to neuroscience goals. This will include methods that already have been demonstrated in vivo, such as 3-photon excitation, and methods that are close to that point today, such as the use of multimode fibers. Research into the more advanced methods (e.g. time-reversed ultrasound) will be initiated in contact with national and international experts (Institut Langevin Paris, Ecole Normale Supérieure, CalTech Biophotonics).

STEP 3-5: EXPANDING RESEARCH AND TRAINING

- During this stage (2019-2024), staff will be appointed to further establish the NeuroPhotonics Institute as on organizational identity, to plan relocation of the different groups and facilities into one central location, and to support research activities.
- Based on the international consultation in Step 2, complementary groups will be selected to join the institute and will be supported with startup budget for staff and equipment. In addition, equipment investments will be made to further support the founding groups and to translate proof-of-principle equipment into facility-type equipment (i.e. more robust and user-friendly).
- To establish cross-disciplinary collaboration and strengthen Dutch neurophotonics, young researchers should be trained at the interface of neurosciences and physics. At the PhD level there are training programs in neurosciences including the Graduate School Neurosciences Amsterdam Rotterdam (ONWAR), the Rudolf Magnus Graduate School of Neuroscience and the Donders Institute for Brain, Cognition and Behaviour (Nijmegen). However, the currently available courses often lack advanced photonics. *Vice versa*, course work at the bachelor, masters and PhD level in Physics are often not addressing Neuroscience applications. The NeuroPhotonics Institute will contribute to more integrated education by establishing courses, workshops, lectures and exchange programs that integrate photonics and neuroscience. Training of young researchers, including PhD students and

early career postdoctoral students will further foster the integration of the NeuroPhotonics research activities in the Netherlands and deliver skilled employees for the photonics industry and research. These training activities will benefit from current efforts of two of the applicants (Mosk and Kapitein) to better integrate physics and biology training at Utrecht University.
- On a technical level, all optical methods developed in step 2 should become operational within the facility in this phase.

STEP 6: ALIGNMENT WITH HIGH-TECH TOP SECTOR

- The proposed facility will need to be strategically aligned with current roadmaps of the High Tech Systems and Materials (HTSM) top-sector[36]. For example, there are obvious links with the photonics cluster, one of the largest backbones of HTSM, which aims to develop photonic devices in diagnostics and therapeutic applications[37]. Our facility, aiming to develop innovative photonic products investigating the complexity of the cellular architecture of the brain, may become an important new partner within the Photonics Roadmap and lead to various mutual benefits. On the one hand, the development of advanced photonic tools for neuroscience research may deliver components with interesting implications for devices for medical diagnostics. On the other hand, the research outcomes of the facility will benefit from the established public-private partnerships that enable marketing of the new knowledge and valorization of newly developed optical and software tools. In addition, some of the NeuroPhotonics activities could also have support from the knowledge valorization experiences within the Roadmap NanoNextNL, aiming to develop and market nanotechnologies in materials and electronic devices, sensors and lightning[38]. Nanotechnologies are part of the techniques that probe the detailed architecture of the brain. These valorization activities within the NeuroPhotonics Institute may be developed along a similar strategy adopted by the NanoNextNL Roadmap, in which several stages of the business plans are examined for aspects of intellectual property, market research and investors (the so called 'Golden Egg Check').

STEP 7-8: A PHYSICAL NEUROPHOTONICS INSTITUTE

- Although the initially virtual NeuroPhotonics Institute will already contribute to in the development of new imaging and recording techniques, the concentration of research and development expertise on a single site has major benefits over a distributed network of research organizations. In the mid-long term there is the need to have short communication lines between physics and neuroscience research groups and between workshop, research labs and facility management. Such cross-disciplinary activities can be best achieved by housing the NeuroPhotonics Institute within one building, removing any physical restrictions. The staff appointed in Step 3, will explore the possible scenarios to achieve this centralized facility in 2024.
- The facility will be operational as described in the technical case (Section B) and combine access to external and internal users with the continuous development of improved or novel instrumentation.
- In connection with the stakeholders in the high-tech sector (Step 6), valorization opportunities will be continuously monitored, potentially resulting in patent applications or startup activities.

[36] Holland Photonics Roadmap, 29 November 2012. (www.rvo.nl/).
[37] http://www.photonicsnl.org/
[38] www.nanonext.nl