

Registration form (basic details)
1a. Details of applicant

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1b. Title of research proposal

Ultra-resolution in optical nanoscopy

1c. Scientific summary of research proposal

Optical nanoscopy is a revolutionary technique used to study biological structure and function at the nanometer scale using fluorescent labels. Localization nanoscopy offers a $\sim 10\times$ better resolution than conventional diffraction-limited microscopy, improving the resolution from ~ 250 to 30 nm. To image structures with molecular-level resolution on the 1 -nm scale, another factor $10\times$ better is needed. This would enable seeing the structure and protein composition of e.g. (macro)molecular assemblies such as the nuclear pore complexes in 3D. The composition of these evolutionary preserved complexes is still much debated but determines their important function as gateway between cell nucleus and cytoplasm and their role in autoimmune diseases.

Fluorescence imaging at cryogenic temperatures enables high localization precision (below 1 nm) as more photons can be acquired from the labels, but the actually achievable 3D resolution is still not in the desired 1 -nm range. This is due to 1) unavoidable incomplete fluorescent labelling and 2) inherent poor axial resolution in microscopy.

I propose a combination of prior-knowledge-driven image reconstruction schemes and a new cryogenic imaging technique to enable for the first time 3D isotropic localization of single molecules with 1 -nm resolution. To this end, I will 1) combine information from many chemically identical complexes into one single reconstruction in a manner that compensates for incomplete fluorescent labelling, and 2) realize an experimental setup and methodology for imaging at cryogenic temperatures with two opposing aberration-corrected objectives. The latter will offer isotropic localization precision in the sub-nm range and improved axial resolution through interferometric detection.

Importantly, the cryogenic fluorescence imaging can be directly followed by cryo-electron microscopy in one workflow. As such, my approach will tap in on advancements in cryo-electron microscopy. Only with the high (fluorescence-based) functional resolution of my method can the full potential of identifying proteins imaged "in grey" with electrons be realized.

1d. Keywords

3D super-resolution microscopy, structural biology, cryo fluorescence, multi-view geometry, image reconstruction

1e. Current institution of employment

Faculty of Applied Sciences
Delft University of Technology
Lorentzweg 1
2628 CJ Delft

1f. Prospective host institution

As above

1g. NWO assessment committee (Choose one)

Applied and Engineering Sciences (AES)	X
Health Research and Development (ZonMw)	
Science Domain (ENW)	
Social Sciences and Humanities (SSH)	
Cross-domain committee (DO)	

1h. Main field of research

Code	Main field of research
16.20.00	Software, algorithms, control systems
	Other fields of research
12.20.00	Nanophysics/technology
14.90.00	Technology, others
21.90.00	Life sciences, other

1i. Public summary of your research proposal
Zooming in on the building blocks of life, one at a time.

Prof.dr. Bernd Rieger (m), TUD – Imaging Physics

Microscopes can probe into biological matter deeper and deeper. Using fluorescent labels, they can even discern single molecules. Researchers solve a persistent shortcoming of fluorescence microscopy and devise a new microscopy methodology to image molecular structures at the millionth of a mm scale, opening new ways to answer fundamental questions about the building blocks of life.

Inzoomen op de bouwstenen van het leven, één voor één.

Prof.dr. Bernd Rieger (m), TUD – Imaging Physics

Microscopen kunnen steeds dieper inzoomen op biologische materialen. Door middel van fluorescerende labels kunnen ze zelfs enkele moleculen onderscheiden. Onderzoekers lossen een hardnekkig tekortkoming van fluorescentiemicroscopie op en ontwikkelen een nieuwe microscopiemethode om moleculaire structuren van een miljoenste mm groot te bekijken, om uiteindelijk fundamentele vragen over de bouwstenen van het leven te kunnen beantwoorden.

Research proposal
2a. Description of the proposed research

Super-resolution microscopy has revolutionized nano-scale imaging by making it possible to image sub-cellular structures at ~ 30 -nm length scales with visible light^{1,2}. The Nobel Prize in chemistry was awarded in 2014 to Hell, Betzig and Moerner for this innovation. A new leap in resolution of a factor of 10 would open up the exciting possibility of imaging *within* the macromolecular complexes that make up the machinery of life.

Single-molecule localization microscopy (SMLM) is the most widely applied type of optical super-resolution microscopy. Its final resolution is however limited by³ **(1)** incomplete fluorescent labelling, which is unavoidable in practice⁴; **(2)** the limited number of photons that can be harvested from a single fluorescent molecule⁵; and **(3)** the resolving power along the microscope's optical axis that is inherently 3-5x worse than in the image plane. I aim to overcome these three connected challenges and obtain *isotropic 1-nm resolution in 3D*.

2a1. Objectives
I. 3D isotropic imaging on the 1-nm scale

Cryogenic imaging offers sub-nanometer localization precision because of the virtually unlimited photon count^{6,7} but lacks ways for on-off switching of fluorophores⁷. In my current ERC-CoG project I use orientational STED-based sparsity⁸ (**Fig. 1b**). I now propose a major step forward by adding a second opposing objective lens and replacing the large, bulky liquid-nitrogen tank^{6,8} with a small cryo-stage to cool the sample, which allows imaging from opposite sides (**Fig. 1a,c**). These innovations enable much better STED polarization control in 3D and thus better sparsity - key to higher resolutions. Additionally, this so-called 4pi-setup collects twice the number of photons, but most importantly offers a ~ 10 x better axial resolution by interferometric detection^{9,10} (**Fig. 1c**). The combination of these innovations will overcome limitations **(2)** and **(3)**. Orientational STED for 1-nm-scale imaging needs extreme polarization control requiring double aberration correction (**Fig. 1c**). I will move from simple "guide star" solutions from astronomy and imaging through thick (whole cells, ~ 10 μ m thick) specimens to calibration and phase diversity protocols tailored to cope with the "frozen" (fixed) emission dipoles¹¹ of the fluorescent labels.

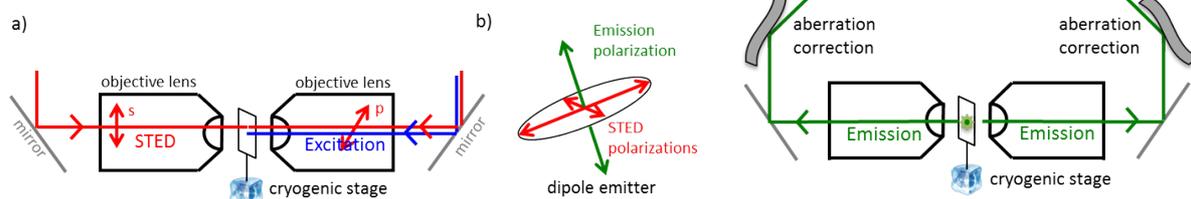


Figure 1: 4pi cryo-microscopy setup for 1-nm-scale resolution.

a) Schematics of a dual-objective setup with excitation of the fluorescent sample on a cryo-stage and STIMULATED EMISSION DEPLETION (STED) from opposing sides; **b)** Emission of fixed dipole emitter is restricted to the polarization perpendicular to the STED polarizations plane to enable sparsity for localization microscopy; **c)** Estimation of the 3D position with nanometer precision with aberration-corrected interferometric detection.

II. Prior knowledge and data fusion for 1-nm-scale resolution

The fusion of multiple acquisitions of chemically identical copies of a biological structure (termed "particle"), e.g. a protein assembly such as the nuclear pore complex, into one hyper-resolved reconstruction can mitigate limitation **(1)**, the incomplete (30-70%) fluorescent labelling that occurs in practice. Such a reconstruction effectively has many more localizations than the individual SMLM images, resulting in a much better signal-to-noise ratio and a resolution improvement. This approach is similar to the concept of single-particle analysis in cryo-electron microscopy^{12,13} (cryo-EM; Nobel prize Chemistry 2017). I have pioneered the adoption of this concept in optical nanoscopy^{14,15}, which has already resulted e.g. in solving a long-standing debate on the composition of the nuclear pore complex¹⁶.

The state-of-the-art data fusion pyramid¹⁵ is depicted in **Fig. 2a** where the registration of two individual particles is performed by cross-correlation-based algorithms copied from EM^{16,17}. However, the image formation in SMLM is completely different. Apart from the incomplete fluorescent labeling, statistical variations in localization uncertainty, false-positive localizations and repeated localizations of the same fluorophore are sources of randomness making the data more complex than in cryo-EM. In addition, any pyramid approach suffers from a large sensitivity to registration errors in the bottom layer, propagating into subsequent layers of the registration.

Here, I propose a particle-fusion approach, which assumes no prior knowledge of the structure to be imaged (template-free), works directly on the localization data (including the uncertainties) without pixilation, and is robust against registration errors and underlabeling.

The key idea is to use an all-to-all registration procedure, in which each particle is registered to all others (**Fig. 2b**). This generates the maximum information that can be extracted. Each pair registration results in the best estimate of the *relative* orientation and position of the two particles. What is needed, however, are the *absolute* orientations and positions of all particles. I propose to solve this by Lie-algebraic averaging, inspired by the computer-vision field "structure from motion"^{18,19}. The high axial resolution obtained by objective I is needed as it determines the overall 3D registration quality. Preliminary 2D results for a fully labelled artificial structure created with DNA-origami (collaboration Jungmann lab^{20,21}) show ~4-nm details, highlighting the potential of my approach (**Fig. 2c**).

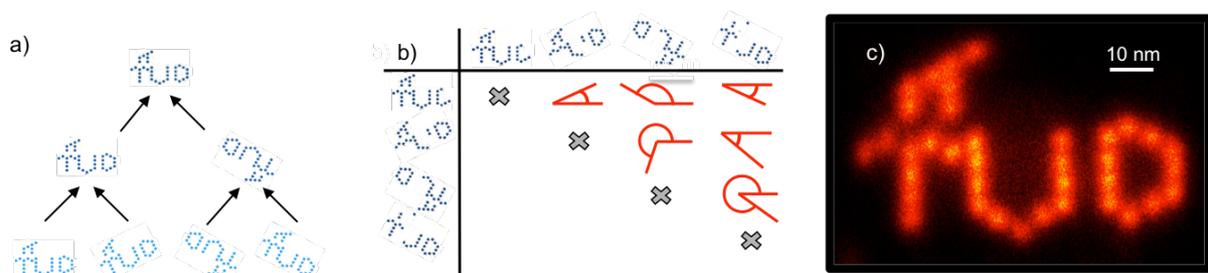
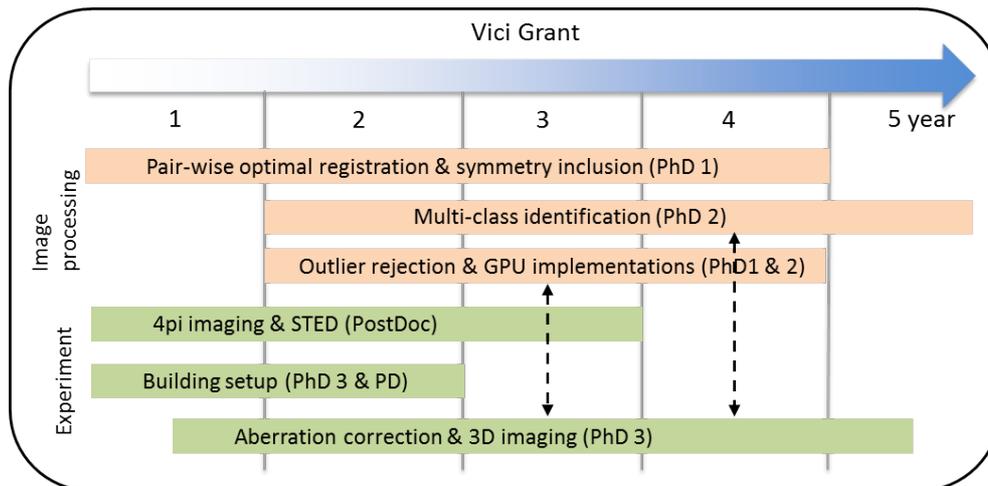


Figure 2: Data fusion in optical nanoscopy for 1-nm-scale resolution. a) State-of-the-art pair-wise registration scheme of "particles" into a single high-resolution particle without a template, **b)** Proposed globally optimal registration by all-to-all alignment resulting in a redundant set of $N(N-1)/2$ relative transformation parameters which can compensate for inherent registration errors, **c)** Preliminary reconstruction result from 456 individual super-resolution 2D PAINT²¹ images of a DNA-origami structure obtaining wavelength/130 resolution [unpublished].

2a2. Research plan

My group has leading expertise in algorithm development and (cryo) super-resolution imaging. For this project, I will assemble a team consisting of 3 PhD students and a PostDoc. The work will be carried out in the Van Leeuwenhoek Laboratory (VLLAIR), a Center of Excellence of TUD which is run by my department, offering suitable low-vibration, high-thermal-stability lab space. The table below shows the timetable over the grant period and the work plan for the requested personnel.



PhD1 and **PhD2** will work together on the algorithmic development of the registration. PhD1 will first work out the best way to align two particles with the inclusion of uncertainties and potential symmetry of the particles. PhD2 will build upon this work and look into automatic classification of a-priori unknown different classes of particles within the data (biological heterogeneity). Both PhDs will tackle common challenges concerning robust outlier rejection and alignment error control and require code optimization for speed on GPU as we still anticipate computation times of hours. They will interact with the experimental team (**PD** and **PhD3**) to collectively enable the breakthrough in structural imaging on the 1-nm scale. PhD3 and PD will work on their respective tasks using a shared optical table and combine their setups once each part is established via proof-of-principle experiments.

Collaborations

I will collaborate intensively with leading international biophysics/technology groups: Markus Sauer (Würzburg), who is recognized as pioneer in designing, understanding and applying fluorescent labels in super-resolution microscopy; Ralf Jungmann (LMU München), who is leading in DNA PAINT-based imaging and DNA nanotechnology; and David Grünwald (U. Massachusetts), leader in imaging single molecules inside the cell nucleus. At TU Delft, I will collaborate with Michel Verhaegen on aberration correction, Arjen Jacobi on thin cryo-sample preparation from cells and biological tissue, and Jacob Hoogenboom on the integration of our stage with his correlative light and EM platform.

Risk assessment

Most critical is the ultra-stable cryo-stage (e.g. <5 nm/min drift). In case it is significantly delayed or instable we fall back to room-temperature imaging with suboptimal caged-dyes or PAINT imaging. The data fusion will still give results albeit at a lower resolution (probably ~5 nm range).

2b. Knowledge utilisation

As an enabling tool, the outcomes of this project will impact the biomedical community as well as provide possibilities for commercialisation by the microscopy industry. Promoting knowledge utilisation is therefore along three lines: (1) Disseminating the outcomes through scientific articles and conference contributions, (2) Commercialising cryo-stage microscopy hardware, and (3) Making available open-source software for the data fusion reconstructions.

On the **hardware** side I will collaborate closely with Delmic BV as commercial partner as they build and market correlative light-electron microscopes and currently have a larger initiative to develop a cryo-stage for their market. I will also collaborate with Demcon-Kryoz, who will supply the micro-cooler (SQUID-based) used in the proposed technology and I have been actively involved in starting the collaboration between Delmic and Demcon-Kryoz. During the development and implementation phase of my cryo-stage, I plan bi-weekly meetings with Delmic to align my design efforts with those by Delmic for correlative microscopy. In this way, my techniques will be directly transferrable to correlative light and electron microscopy application.

The developed setup will be installed in the Van Leeuwenhoek Laboratory (VLLAIR) at TU Delft, a lab specifically designed for collaboration with end-users and industrial partners. As Jacob Hoogenboom also hosts his research at the VLLAIR and as he is working on correlative light and electron microscopy in collaboration with Delmic, we expect a mutual beneficial environment for sharing of techniques.

On the **software** side I will distribute the new algorithms open-source, such that other *developers* (not biological end-users) can use our findings freely and without time loss to recode our algorithms. For the dissemination I will continue to use our image processing library DIPimage (www.diplib.org). I have been one of its principal architects and now, as scientific director of the project, initiated a recoding from scratch of the 20-years old code. For end-users in the biology community I will additionally provide ImageJ plugins for maximal impact. These efforts constitute a significant amount of work (commenting, cross-platform and version testing but foremost maintenance), but in my experience it is rewarding not only as service to the community but also as starting point for new collaborations later on.

I will use the new software on data provided by my current collaborators, including UvA, ErasmusMC and NKI, which will demonstrate applicability for a wide variety of use cases and help dissemination to a wide research community.

My track record of software transfer to academic and industrial partners includes work on defocus estimation²², which has been transferred to FEI and is now integrated in their TEM release software. On the end-user side, early involvement in algorithm development resulted in a number of co-publications where the software could be directly coupled to biological questions^{14,23,24}.

The following users have already agreed to participate in the user committee (meeting twice a year on-site): Peter Drent (CEO confocal.nl), Sander de Hoedt (CEO Delmic), Raimond Ravelli (correlative light and electron microscopy, Maastricht University), Lukas Kapitein (cellular biophysics, Utrecht University), Erik Meijering (biomedical image processing, ErasmusMC).

2c. Number of words used

section 2a1 and 2a2: 1200 (max. 1,200 words)

section 2b: 495 (max. 500 words)

2d. Literature references

- [1] S.W. Hell. Microscopy and its focal switch. *Nature Methods*, 6:24-32, 2009.
- [2] T. Klein, S. Proppert, M. Sauer. *Eight years of single-molecule localization microscopy*. *Histochem Cell Biology*, 141(6):561-575, 2014.
- [3] R.P.J. Nieuwenhuizen, K.A. Lidke, M. Bates, D. Leyton Puig, D. Grünwald, S. Stallinga, **B. Rieger**. *Measuring image resolution in optical nanoscopy*. *Nature Methods*, 10(6):557-562, 2013.
- [4] M. Sauer and M. Heilemann. *Single-molecule localization microscopy in eukaryotes*. *Chemical Reviews*, 117:7478-7509, 2017.
- [5] G.T. Dempsey, J.C. Vaughan, K.H. Chen, M. Bates, X. Zhuang. *Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging*. *Nature Methods*, 8(12):1027-1031, 2011.
- [6] W. Li, S.C. Stein, I. Gregor, J. Enderlein. Ultra-stable and versatile widefield cryo-fluorescence microscope for single-molecule localization with sub-nanometer accuracy. *Optics Express*, 23(3):3770-3783, 2015.
- [7] S. Weisenburger, D. Boening, B. Schomburg, K. Giller, S. Becker, C. Griesinger, V. Sandoghdar. Cryogenic optical localization provides 3D protein structure data with Angstrom resolution. *Nature Methods*, 14(2):141-144, 2017.
- [8] C. Hulleman, M. Huisman, R. Moerland, D. Grünwald, S. Stallinga, **B. Rieger**, Fluorescence polarization control for on-off switching of single molecules at cryogenic temperatures, *Small Methods*, 2018 (bioRxiv doi.org/10.1101/204776).
- [9] G. Shtengel, J.A. Galbraith, C.G. Galbraith, J. Lippincott-Schwartz, J.M. Gillette, S. Manely, R. Sougrat, C.M. Waterman, P. Knachanawong, M.W. Davidson, R.D. Fetter, H.F. Hess. *Interferometric fluorescent super-resolution microscopy resolves 3D cellular ultrastructure*. *Proceeding of the National Academy of Science USA*, 106(9):3125-3130, 2009.
- [10] F. Huang, G. Sirinakis, E.S. Allgeyer, L.K. Schroeder, W.C. Duim, E.B. Kromann, T. Phan, F.E. Rivera-Molina, J.R. Myers, I. Irnov, M. Lessard, Y. Zhang, M.A. Handel, C. Jacobs-Wagner, C.P. Lusk, J.E. Rothman, D. Toomre, M.J. Booth, J. Bewersdorf. *Ultra-high resolution 3D imaging of whole cells*. *Cell*, 166:1-13, 2016.
- [11] S. Stallinga and **B. Rieger**. *Position and orientation estimation of fixed dipole emitters using an effective Hermite point spread function model*. *Optics Express*, 20(6):5896-5921, 2012.
- [12] F.K.M. Schur, W.J.H. Hagen, A. de Marco, J.A.G. Briggs. *Determination of protein structure at 8.5 Å resolution using cryo-electron tomography and sub-tomogram averaging*. *Journal of Structural Biology*, 184(3):394-400, 2013.
- [13] J. Kosinski, S. Mosalaganti, A. von Appen, R. Teimer, A.L. DiGuilio, W. Wan, K. Huy Bui, W.J.H. Hagen, J.A.G. Briggs, J.S. Glavy, E. Hurt, M. Beck. *Molecular architecture of the inner ring scaffold of the human nuclear pore complex*. *Science*, 352(6283):363-365, 2016.
- [14] Löscherberger, S. van de Linde, M.C. Dabauvalle, **B. Rieger**, M. Heilemann, G. Krohne, M. Sauer. *Super-resolution imaging visualizes the eightfold symmetry of gp210 proteins around the nuclear pore complex and resolves the central channel with nanometer resolution*. *Journal of Cell Sciences*, 125(3):570-575, 2012.
- [15] J. Broeken, H. Johnson, D.S. Lidke, S. Liu, R.P.J. Nieuwenhuizen, S. Stallinga, K.A. Lidke, **B. Rieger**. *Resolution improvement by 3D particle averaging in localization microscopy*. *Methods and Applications in Fluorescence*, 3:014003, 2015.
- [16] A. Szymborska, N. Marco, A. de. Daigle, V.C. Cordes, J.A.G. Briggs, J. Eillenber. *Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging*. *Science*, 341:655-658, 2013.
- [17] R.D.M. Gray, C. Beerli, P.M. Pereira, K.M. Scherer, J. Samolej, C.K.E. Bleck, J. Mercer, R. Henriques. *Virusmapper: open-source nanoscale mapping of viral architecture through superresolution microscopy*. *Scientific Reports*, 6:29132, 2016.
- [18] F. Kahl and R. Hartely. *Multiple-view geometry under the L_∞ -norm*. *IEEE Transactions on Pattern Analysis and Machine Learning*, 30(9):1603-1617, 2008.

**Vernieuwingsimpuls
Innovational Research Incentives Scheme
Grant application form (pre-proposal) 2018**

Ultra-resolution in optical nanoscopy

Vici scheme

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- [19] V.M. Govindu and A. Pooja. *On averaging multiview relations for 3D scan registration*. IEEE Transactions on Image Processing, 23(3):1289-1302, 2014.
 - [20] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld, and F.C. Simmel. *Single-molecule kinetics and super-resolution microscopy by fluorescence imaging of transient binding on DNA origami*. Nano Letters, 10(11):4756-4761, 2010.
 - [21] J. Schnitzbauer, M.T. Strauss, T. Schlichthaerle, F. Schueder, and R. Jungmann. *Super-resolution microscopy with DNA-PAINT*. Nature Protocols, 12:1198-1228, 2017.
 - [22] M. Vulovic, E. Franken, R.B.G. Ravelli, L.J. van Vliet, **B. Rieger**. *Precise and unbiased estimation of astigmatism and defocus in transmission electron microscopy*. Ultramicroscopy, 116:115-134, 2012.
 - [23] C.S. Smith, S. Preibisch, A. Joseph, S. Abrahamsson, **B. Rieger**, E. Myers, R.H. Singer, D. Grünwald. *Nuclear accessibility of β -actin mRNA measured by 3D single molecule real time (3D-SMRT) microscopy*. Journal of Cell Biology, 209(4):609-619, 2015.
 - [24] R.P.J. Nieuwenhuizen, L. Nahidiazar, E.M.M. Manders, K. Jalink, S. Stallinga, **B. Rieger**. *Co-orientation: Quantifying simultaneous co-localization and orientational alignment of filaments in light microscopy*. PLoS ONE, 10(7):e0131756, 2015.

2e. Data management

1. Will data be collected or generated that are suitable for reuse?

The data that will be handled in this project can be subdivided in two categories:

- i) Simulation image data, used to demonstrate and validate the performance of data fusion algorithms. These data will be made available with the publications resulting from this project, along with the source code.
- ii) Experimental super-resolution images, these are saved on our microscopy server, transferred to internal TU Delft (raid)servers subject to daily backups.

2. Where will the data be stored during the research?

All simulation data will be stored on internal TU Delft servers and will be transferred to a publicly available ftp server upon publication of the results based on this data. All experimental data that can be reused by others, including all data used in publications will be stored on TU Delft data repository.

3. After the project has been completed, how will the data be stored for the long-term and made available for the use by third parties? To whom will the data be accessible?

Along with all publications from this project, we will provide open-source code that can be used to re-generate the identical simulation data reported in publications, thereby strongly reducing the need to store large amounts of simulation data over a long time period. The experimental data gathered in the project will be securely stored at 3TU.Datacentrum (<http://datacentrum.3tu.nl>).

4. Which facilities (ICT, (secure) archive, refrigerators or legal expertise) do you expect will be needed for the storage of data during the research and after the research? Are these available?*

The required ICT infrastructure is present at TU Delft for storing during the research. Later transfer to the 3TU.Datacentrum is foreseen. TU Delft ftp servers can function to maintain long-term access to source code (as is the case for 15+ years for our scientific Image Processing Library DIPlib/DIPimage via www.diplib.org). Together with Erik Meijering (Erasmus MC) I am involved in the Data Management node of NL-bioimaging which is currently under review for bridge financing of the NWO roadmap for large infrastructure in the Netherlands.

Cost estimates

3a. Budget (in k€)

	Description			Year 1	Year 2	Year 3	Year 4	Year 5	Total
	Staff	FTE	Months						
WP	Applicant	0.4	60	45	48	50	52	54	249
WP	PhD1	1.0	48	42	49	52	56		199
WP	PhD2	1.0	48		42	49	52	56	199
WP	PhD3	1.0	48	42	49	52	56		199
WP	PostDoc	1.0	36	60	64	67			191
Total Staff		4.4	240	189	252	270	216	110	1037
Equipment	Dedicated multi-GPU server			30					30
Equipment	Cryo stage			95					95
Equipment	4pi microscope components, table & 2 lasers			120					120
Equipment	2 sCMOS cameras			30					30
Equipment	Double aberration correction			80					80
Materials	Optical components			10	8	5	5	2	30
Materials	Glass/plastic/chemicals			2	2	2	2	2	10
Materials	Electronics/automation			10	8	5	5	2	30
Travel	Applicant			3	3	3	3	3	15
Travel	PhDs & PostDoc			6	8	8	6	2	30
Other	Publication costs				2	3	4	4	13
Total Materials				366	31	26	25	15	463
Grand total				555	283	296	241	125	1520

3b. Contributions 'in kind'

Co-financer/party	Description	Estimated value in euros
Delmic BV	Hardware and software development for cryo-stage	20.000

3c. Contributions 'in cash'

None.

**Vernieuwingsimpuls
Innovational Research Incentives Scheme
Grant application form (pre-proposal) 2018**

Ultra-resolution in optical nanoscopy

Vici scheme

Bernd Rieger

3d. Totals

Grand total	1.520.000
Budget requested from NWO	1.500.000

3e. Have you applied for any additional grants for this project either from NWO or from any other institution, and/or has the same idea been submitted elsewhere?

No.

Curriculum vitae
4a. Personal details

Title(s), initial(s), first name, surname: Prof. Dr. B. (Bernd) Rieger

4b. Master's degree ('Doctoraal')

University/College of Higher Education: Technische Universitat Munchen

Date (dd/mm/yy): 16/07/1999

Main subject: Physics

4c. Doctorate

University/College of Higher Education: Technische Universiteit Delft

Starting date (dd/mm/yy): 01/10/1999

Date of PhD award (dd/mm/yy): 09/02/2004

Supervisor ('Promotor'): Prof.dr.ir. L.J. van Vliet

Thesis title: Structure from Motion in nD Image Analysis

4d. Work experience since completing your PhD

Position	Period	FTE	Position	Institution
Professor*	01/2017-	1.0	Permanent	TU Delft
Associate Professor	06/2014-12/2016	0.8	Permanent	TU Delft
Assistant Professor	05/2006-05/2014	0.8	Tenure track	TU Delft
Senior Researcher	05/2006-12/2010	0.2	Permanent	FEI Company, Eindhoven
Senior Researcher	05/2005-04/2006	1.0	Permanent	FEI Company, Eindhoven
Post-doc	01/2004-04/2005	1.0	Fixed term	MPI for Biophysical Chemistry, Gottingen (NWO TALENT scholarship)

* I am appointed '**Antoni van Leeuwenhoek professor**' which is an honorary early-promotion position for young outstanding researchers at TU Delft.

Months spent since completing your PhD

Experience	Number of months
Research activities	90.52
Education	34.74
Care or sick leave	14.40 (taking care of my children for 1d/week)
Management tasks	6.54
Other	22.80 (working for FEI Company)

Calculation:

 Research: $1.0 \cdot 16 \cdot 100\% + 0.8 \cdot 126 \cdot 65\% + 1.0 \cdot 15 \cdot 60\% = 90.52$

 Education: $1.0 \cdot 16 \cdot 0\% + 0.8 \cdot 126 \cdot 30\% + 1.0 \cdot 15 \cdot 30\% = 34.74$

 Management: $1.0 \cdot 16 \cdot 0\% + 0.8 \cdot 126 \cdot 5\% + 1.0 \cdot 15 \cdot 10\% = 6.54$

 Child care: $0.2 \cdot 72 = 14.40$

 Other: $1.0 \cdot 12 + 0.2 \cdot 54 = 22.80$

Total: 169 months ~ 14y

4e. Academic staff supervised

	Give names or numbers	Please indicate/specify your role (for PhDs, mark <u>one</u> role)		
		Promotor (formal supervisor)	Co-promotor (formal co-supervisor)	Role as (co-) supervisor
PhDs				
Ongoing	<i>Christiaan Hulleman</i>	X		
	<i>Yan Guo</i>	X		
	<i>Hamid Heydarian</i>	X		
	<i>Rasmus Thorsen</i>	X		
Successfully completed	<i>Nadya Chakrova</i>		X	
	<i>Carlas Smith</i>		X	
	<i>Robert Nieuwenhuizen</i>		X (cum laude)	
	<i>Lennart Voortman</i>		X	
	<i>Sanneke Brinkers</i>		X	
	<i>Heidi Dietrich</i>		X	
	<i>Milos Vulovic</i>		X	
<i>Subtotal PhDs</i>	11	4	7	0
Postdocs				
Ongoing	<i>Taylor Hinsdale</i>	<i>Supervisor</i>		
	<i>Robert Moerland</i>	<i>Supervisor</i>		
Completed	<i>Richard Aveyard</i>	<i>Supervisor</i>		
	<i>Vincent van Ravesteijn</i>	<i>Supervisor</i>		
	<i>Melanie Kessels</i>	<i>Supervisor</i>		
<i>Subtotal postdocs</i>	5			
Support staff members				
<i>Subtotal support staff</i>	5	<i>Support staff supervisor</i>		

4f. Brief summary of your research over the last five years

At TU Delft, I lead a research team of 6 researchers working on *Computational Microscopy* in light and electron microscopy. It comprises the combination of imaging physics and image processing to surpass fundamental limitations imposed by physics on image formation. My main application area is in life sciences at the molecular level, with the most notable research activity at the moment in localization or super-resolution microscopy, an area also known as optical nanoscopy.

My team aims to develop techniques that offer the highest spatial (and temporal) resolution in microscopic imaging. With this aim, we place ourselves in between purely curiosity and application-driven research. In the framework of my ERC Consolidator Grant project we are currently developing experimental and theoretical means to enable localization microscopy at cryogenic temperature.

In 2013, I introduced a novel resolution concept and measure for nanoscopy. This measure is now the baseline in the field and used e.g. in the "localization challenge" (Chenouard *et al.*, *Nature Methods* 2014). I provided code to accelerate dissemination and gave many invited talks on the subject.

In 2012, I introduced the concept of "data fusion" to the field of localization microscopy. This technique allows sub-nanometer precision measurements with light on reconstructions from many chemically identical entities. This idea has been used to resolve a long-standing debate about the chemical composition of the Nuclear Pore Complex that eluded cryo-electron microscopy (Szyborska *et al.*, *Science* 2013). My Vici project will lift this concept from its fledgling stages to a mature field.

4g. International activities

International Conferences and Workshops:

- Initiator and Organizer of the conference series *Quantitative Bioimaging* since 2013 www.quantitativebioimaging.com. Each year we attract ~150-200 attendees, ~15 international recognized invited speakers. 2013 & 2014 in Albuquerque, USA; 2015 at Institute Pasteur, Paris, France and 2016 at TU Delft. For Delft we attracted W.E. Moerner as keynote speaker, Noble prize laureate 2014 in chemistry. (2017 in Texas, USA and 2018 in Göttingen, Germany)
- Associate Editor for International Symposia on Biomedical Imaging ISBI 2016, 2017 & 2018, abstract and short paper selection
- Organizer of the "localisation challenge" for single-molecule localization microscopy benchmarking, EPFL 2015
- Keynote lecture at European Bioimaging Conference Eubias, Paris, France, January 5-6, 2015
- Organizer and Lecturer on *Localization Microscopy* at the 22nd Int. Conf. on Pattern Recognition ICPR 2014, Stockholm, Sweden

International Research Activities:

- Visiting scientist for six (2007) and four weeks (2010) at the Department of Physics and Astronomy, University of New Mexico at Albuquerque, USA
- Member of COST action CA15124 - *A new Network of European BioImage Analysts to advance life science imaging (NEUBIAS)*. I have been part of and supported the application process from the beginning; I'm not in a chairing positing right now.

Major International Collaborations:

- Prof.dr. Ralf Jungmann on *Data fusion for 2D and 3D DNA-origami data*, MPI for Biochemistry, Martinsried, Germany. Data exchange and visits.
- Prof.dr. Keith Lidke on *Image analysis in super-resolution microscopy*, Department of Physics and Astronomy, University of New Mexico at Albuquerque, USA. We exchanged in total 3 graduated and 3 undergraduate students for each ~3 months stay over the years. I visited several times and prof. Lidke stayed 2 months in Delft in spring 2017 for a short sabbatical.
- Prof.dr. Markus Sauer on *Algorithms for counting in super-resolution microscopy*, Biozentrum, University of Würzburg, Germany. Several short visits (1-2 days) over the last years.
- Prof.dr. David Grünwald on *Image analysis for very weak signals from single molecules emitters*, University of Massachusetts Medical School Worcester, USA. We exchanged 3 students each for several months over the last year and prof. Grünwald visited Delft a couple of times in the last years. We had one shared PhD student. We currently jointly lead NIH-funded research on 4D chromatin structure.

Invited Talks (selection of 33 thus far):

- 2nd International Conference On Nanoscopy, ICON, Bielefeld, Germany, February 27 – March 2, 2018
- 19th Linzer Winterschool on Biophysics, Linz, Austria, February 5-7, 2017
- SelectBio: Bioimaging From Cells To Molecules, Cambridge, UK, June 14-15, 2016
- European Bioimaging Conference Eubias, Paris, France, January 5-6, 2015 (*keynote lecture*)
- Royal Society Workshop on Super-resolution microscopy, Leeds, UK, July 3-4, 2014
- 3rd Single Molecule Localization Microscopy Symposium, Frankfurt, Germany, August 28-30, 2013

4h. Other academic activities

Organisational Responsibilities:

- Head of section Quantitative Imaging at TU Delft - since 2018
- Board member and treasurer, Dutch Microscopy Society (NVVM) - since 2010
- Board member and treasurer, Foundation for the promotion of Electron Microscopy in the Netherlands (SEN) - since 2010
- Member of the Career Committee, Faculty of Applied Sciences, TU Delft – since 2018
- Member of the TU Delft Diversity Team (established 2018)
- Member of the Curriculum Committee Applied Physics, TU Delft – since 2014
- Member of the Examination Committee Applied Physics, TU Delft – 2012-2014
- Chairman of the Departmental Safety Committee, TU Delft – 2006-2009
- Organizer of the focus session on Single Molecule Microscopy at the Physics@FOM national conference in Veldhoven, The Netherlands - January 2014
- Serving on external Ph.D. committees in Netherlands; opponent in Uppsala, Sweden

Reviewing:

- Regular reviewer for many journals (identified as one of the best reviewers for Nature Publishing Group 2015).

- External expert reviewer for trans/national funding agencies: STW, STW HTSM jury, NWO Veni/Vidi; Wageningen University (VLAG Graduate School); Wellcome Trust (UK) for the Collaborative Award and Technology Development Grant; Swiss National Science Foundation (SNSF) for the SNSF Professorships (comparable to Vici); Austrian Science Fund (FWF) for the Open Programmes and START Programme (comparable to NWO-Vidi); German Carl-Zeiss Stiftung Jena (comparable to NWO-Veni); Agence Nationale de la Recherche (AAP ANR, France); Fonds Wetenschappelijk Onderzoek – Vlaanderen (FWO, Belgium); Deutsche Forschungsgesellschaft (DFG); Human Frontier Science Programme (HFPS) for research grants

Outreach (selected):

- Lunch lecture on the Noble prize in Chemistry 2014 for physics students, Vereniging voor Technische Physica; *350 years of light microscopy in Delft*, Family day of the Faculty of Applied Sciences, TU Delft, 2016;
- Teaching for high-school children (4 days), International School of The Hague, 2012 & 2013;
- Developed an animation on super-resolution microscopy (2017)
http://www.youtube.com/watch?v=vh3_qOy2uls&t=5s

Valorisation:

- FEI Company (Eindhoven) implemented my defocus estimation algorithms [Vulović et al. Ultramicroscopy 2012] into their release Electron Microscope analysis software, *AutoCTF*;
- Mapper Lithography (Delft) uses my fast GPU implementation [Smith2010] for spot estimation for auto-alignments of wafers
- Consultant for FEI Company and ALSI, Beuningen (now ASM Pacific Technologies)

Undergraduate Teaching and course material development (selected):

- *System and Signals*, 6 ECTS, mandatory course ~300 Applied Physics & Nanobiology students – since 2006; Principal Instructor since 2010
- *Advanced Digital Image Processing*, 6 ECTS, elective MSc, ~50 students from interdisciplinary engineering backgrounds, since 2017
- *Applied Physics Research labs*, second year Applied Physics: I created the Super-Resolution Microscopy lab programme from scratch – since 2015
- *Computational Science*, 3 ECTS, mandatory course ~250 Applied Physics & Nanobiology students, including a major renewal of the course material – 2007-2015; Principal Instructor 2009-2015
- *Computational Science*, minor Applied Physics, 3 ECTS, mandatory course ~35 students, Principal Instructor incl. course material development – 2009-2015
- *High Resolution Microscopy*, 4 ECTS, mandatory course ~20 Nanobiology students, incl. course material development – since 2015
- *Introduction to Imaging Systems*, 3 ECTS, elective course ~80 Applied Physics students

Innovation in Education:

- Acquired a TU Delft 'Studiesucces' grant of 3000€ for developing formative digital homework via eLearning for the *Systems and Signals* course module – 2014/2015
- Acquired grassroots funds worth 1000€ to implement the eClicker system towards active student participation during lectures in the *Systems and Signals* course module - 2012

**Vernieuwingsimpuls
Innovational Research Incentives Scheme
Grant application form (pre-proposal) 2018**

Ultra-resolution in optical nanoscopy

Vici scheme

Bernd Rieger

Supervision of Bachelor/Master's student projects:

- Supervised 11 MSc and 18 BSc student thesis projects. R.P.J. Nieuwenhuizen received the biannual prize for best Applied Physics MSc. thesis 2011 and C.S. Smith for the best BSc. thesis 2008.

Graduate Teaching:

- *Biophysics: measuring and modeling biology*, one-week course, French Biophysical Society and the French Microscopy Society, Chamonix, France - 2015
- *Advanced Microscopy*, Casimir Graduate School, Delft and Leiden – since 2013
- *Advanced Microscopy*, two-week EMBO course for two weeks, Buenos Aires, Argentina - 2006

4i. Scholarships, grants and prizes

Scholarship/Grant/Prize as formal applicant	Total amount	Amount allocated to my research	Year of award
<i>eScience Center, Pathfinder project</i>	50 k€	50 k€	2016
<i>ERC Consolidator Grant; Optical nanoscopy at 1 nm resolution: far-field fluorescence control at cryogenic temperatures</i>	2000 k€	2000 k€	2015
<i>STW-HTSM, Electron Tomography for next generation Integrated Circuits</i>	760 k€	400 k€	2013
<i>NWO middelgroot, Super-resolution in optical sections</i>	174 k€	174 k€	2008
<i>NWO talent fellowship, Quantitative Imaging for Molecular Biology</i>	30 k€	30 k€	2004
Scholarship/Grant/Prize as formal co-applicant			
<i>National Institute of Health, NIH U01, 4DN extension grant</i>	660 k\$	180 k\$	2017
<i>National Institute of Health, NIH U01, 4DN, Visualizing local and global chromatin architecture, and gene expression in individual cells by structural single-molecule imaging</i>	1000 k\$	350 k\$	2015
<i>STW-Perspective; Super-resolution microscopy</i>	5600 k€	1200 k€	2012
<i>BSIK/CONDOR; Image Processing for Scanning Electron Microscopy</i>	2500 k€	290 k€	2010
<i>FOM-FEI-IPP; Ultra-resolution in 3D-cryo TEM</i>	2500 k€	590 k€	2009

Output

5a. Output indicators

My research field is highly interdisciplinary. I am active in the development of theory, algorithms and applications related to *computational microscopy*, with my main focus on *optical nanoscopy in molecular cell biology*. In all of these fields, the main output indicators are peer-reviewed publications in journals and conferences and citations thereof.

My publications span across many domains. Depending on the type of research work, results can be published in engineering journals, physics journals, computer science journals and a range of applied journals (mostly molecular biology).

In summary, my work thus far has been cited 2604 times (*h*-index 25, *i10*-index 46) according to Google Scholar.

Specifically in the domain of image processing, pre-reviewed conference proceedings are also valued and highly competitive. I therefore do not give impact factors.

Finally, in my field, software methods and development are also important. Making them freely available to users in a suitable fashion is a prerequisite for impact.

I have been providing output in the format of *DIPlib/DIPimage* since 2000. *DIPimage* is a MATLAB toolbox for scientific image processing and analysis based on the C-library *DIPlib* (www.diplib.org, 1000+ downloads/year); I know of at least 20 academic groups actively using this software. Providing free software and maintaining a large package is a costly and time consuming enterprise not "rewarded" in citations typically, but very important for the community. Therefore I provide free implementations of all of my algorithms from publications in MATLAB, but also GPU code or ImageJ plugins where appropriated.

5b. Top publications

Number of citations (Google Scholar) denoted between brackets. **[S]** = significant to the current proposal. Full publication list: <http://homepage.tudelft.nl/z63s8/publications>

- 1) **Resolution improvement by 3D particle averaging in localization microscopy [S]**
 J. Broeken, H. Johnsson, D.S. Lidke, L. Sheng, R.P.J. Nieuwenhuizen, S. Stallinga, K.A. Lidke, B. Rieger
Methods and Applications in Fluorescence, 3:014003, 2015. [19]
 Invited contribution to this special issue. In 2016 it was highlighted by the journal in a compilation of excellent papers published in the journal. Developed a framework for enabling 3D data fusion in fluorescence microscopy. Initiated and directed the research, lead the collaboration between Delft and USA.
- 2) **Measuring image resolution in optical nanoscopy [S]**
 R.P.J. Nieuwenhuizen, K.A. Lidke, M. Bates, D. Leyton Puig, D. Grünwald, S. Stallinga, B. Rieger
Nature Methods, 10(6): 557-562, 2013. [211]
 Found a way to assess resolution in optical nanoscopy; provided a software solution that is currently widely used. Initiated and directed the research, lead the collaboration between Delft, Amsterdam, USA and Germany.
- 3) **Image formation modeling in Cryo Electron Microscopy**
 M. Vulović, R.B.G. Ravelli, L.J. van Vliet, A.J. Koster, I. Lazić, U. Lücken, H. Rullgård, O. Öktem, B. Rieger
Journal of Structural Biology, 183(1):19-32, 2013. [33]
 Developed a simulation framework and validated that with cryo-EM experiments; provided software package InSilicoTEM. Initiated and directed the research, lead the collaboration between Delft, Leiden, FEI Company and Stockholm.
- 4) **Fast, single-molecule localization that achieves theoretically minimum uncertainty**
 C.S. Smith, N. Joseph, B. Rieger*, K.A. Lidke* **[S]**
Nature Methods, 7(5):373-375, 2010. [326]
 First to show that theoretical optimal fitting can be done in real time; provided a software solution that is currently much used. Initiated the research together with K. Lidke, sent my undergrad student C. Smith to work in his lab on the problem for 3 months.
- 5) **Superresolution by localization of quantum dots using blinking statistics**
 K.A. Lidke*, B. Rieger*, T.M. Jovin, R. Heintzmann
Optics Express, 13(18):7052-7062, 2005. [310]
 Found the algorithmic breakthrough for separating quantum dots positions from their intermittent emission. Keyed the term pointillism for the image formation.

5c. Output (selection)

- 6) **[S]** C. Hulleman, M. Huisman, R. Moerland, D. Grünwald, S. Stallinga, B. Rieger, *Fluorescence polarization control for on-off switching of single molecules at cryogenic temperatures*, *Small Methods*, accepted, 2018.
- 7) K. Martens, A.N. Bader, S. Baas, B. Rieger, J. Hohlbein, *Phasor based single-molecule localization microscopy in 3D (pSMLM-3D): an algorithm for MHz localization rates using standard CPUs*, *Journal of Chemical Physics*, 148, 2018.
- 8) R. Aveyard, Z. Zhong, K.J. Batenburg, B. Rieger, *Optimizing experimental parameters for the projection requirement in HAADF-STEM tomography*,

- Ultramicroscopy, 177:84-90, 2017. [1]
- 9) N. Chakrova, B. Rieger, S. Stallinga, *Deconvolution methods for structured illumination microscopy*, Journal of the Optical Society of America A, 33, 2016. [16]
 - 10) C.S. Smith, S. Preibisch, A. Joseph, S. Abrahamsson, B. Rieger, E. Myers, R.H. Singer, and D. Grünwald, *Nuclear accessibility of β -actin mRNA measured by 3D single molecule real time (3D-SMRT) microscopy*, J. Cell Biology, 209, 2015. [20]
 - 11) [S] B. Rieger, S. Stallinga, *The lateral and axial localization uncertainty in super-resolution light microscopy*, ChemPhysChem, 15, 2014. [41]
 - 12) [S] L.M. Voortman, M. Vulović, M. Maletta, A. Voigt, E.M. Franken, A. Simonetti, P.J. Peters, L.J. van Vliet, B. Rieger, *Quantifying resolution limiting factors in subtomogram averaged cryo-electron tomography using simulations*, J. Structural Biology, 187, 2014. [13]
 - 13) M. Vulović, L.M. Voortman, L.J. van Vliet, B. Rieger, *When to use the projection assumption and the weak-phase object approximation in phase contrast cryo-EM*, Ultramicroscopy, 136, 2014. [25]
 - 14) [S] S. Stallinga, B. Rieger, *Position and orientation estimation of fixed dipole emitters using an effective Hermite PSF model*, Opt. Exp., 20, 2012. [29]
 - 15) [S] A. Löscherberger, S. van de Linde, M.-C. Dabauvalle, B. Rieger, M. Heilemann, G. Krohne, M. Sauer, *Super-resolution imaging reveals eightfold symmetry of gp210 proteins around the nuclear pore complex and resolves the central channel with nanometer resolution*, J. Cell Science, 125, 2012. [159]
 - 16) L.M. Voortman, S. Stallinga, R.H.M. Schoenmakers, L.J. van Vliet, B. Rieger, *A fast algorithm for computing and correcting the CTF for tilted, thick specimens in TEM*, Ultramicroscopy, 111:1029-1036, 2011. [20]
 - 17) [S] S. Stallinga, B. Rieger, *Accuracy of the Gaussian point spread function model in 2D localization microscopy*, Optics Express, 18, 2010. [120]
 - 18) D.S. Lidke, F. Huang, J.N. Post, B. Rieger, J.L. Thomas, J. Pouyssegur, T.M. Jovin, P. Lenormand, *ERK nuclear translocation is dimerization-independent but controlled by the rate of phosphorylation*, J. Bio. Chem., 285, 2010. [76]
 - 19) S. Brinkers, H.R.C. Dietrich, F.H. de Groote, I.T. Young, B. Rieger, *The Persistence Length of Double Stranded DNA Determined Using Dark Field Tethered Particle Motion*, J. Chemical Physics, 130, 2009. [81]
 - 20) F.G.A Faas*, B. Rieger*, L.J. van Vliet, D. Cherny, *DNA deformations near charged surfaces: electron and atomic force microscopy views*, Biophys. J., 97, 2009. [23]
 - 21) D.S. Lidke, K.A. Lidke, B. Rieger, T.M. Jovin, D.J. Arndt-Jovin, *Reaching out for signals: Filopodia act as sensory organs via retrograde transport of activated EGF receptors*, J. Cell Biology, 170, 2005. [222]
 - 22) K.A. Lidke*, B. Rieger*, D.S. Lidke, T.M. Jovin, *The role of photon statistics in fluorescence anisotropy imaging*, IEEE Trans. Image Processing, 14, 2005. [63]
 - 23) B. Rieger, F.J. Timmermans, L.J. van Vliet, P.W. Verbeek, *On curvature estimation of surfaces in 3D grey-value images and the computation of shape descriptors*, IEEE Trans. Pattern Analysis and Machine Intelligence, 26, 2004. [46]
 - 24) B. Rieger, L.J. van Vliet, *Curvature of n-dimensional Space Curves in Grey-value Images*, IEEE Trans. Image Processing, 11, 2002. [30]

Total numbers of output items:

- | | |
|---------------------------------|--|
| - Refereed articles: | 66 |
| - Non-refereed articles: | 5 (e.g. Nederlandse Tijdschrift voor Natuurkunde) |
| - Book chapters: | 2 |
| - Patents: | 4 (including an ISO standard ISO/TS 24597:2011) |

5d. Median impact factors for your field: N/A

Statements by the applicant

Use of extension clause: no

Ethical aspects

	Not applicable	Not yet applied for	Applied for	Received
Approval from a recognised (medical) ethics review committee	X			
Approval from an animal experiments committee	X			
Permission for research with the population screening Act	X			

If applicable, proof of approval will have to be sent to NWO before the start of your Vici project.

Declarations

By submitting this form, I endorse the code of conduct for laboratory animals and the code of conduct for biosecurity/possibility for dual use of the expected results and will act accordingly if applicable.

- I have completed this form truthfully.
- By submitting this document I declare that I satisfy the nationally and internationally accepted standards for scientific conduct as stated in [the Netherlands Code of Conduct for Scientific Practice 2012](#) (Association of Universities in the Netherlands).
- I have submitted non-referees.¹

Name: Bernd Rieger

Place: Delft

Date: 26 March 2018

¹ You may indicate up to three non-referees in ISAAC or, for applications submitted to the ZonMw domain, directly to vici@zonmw.nl. Note that, while there is room for five names in ISAAC, you may only list three. The non-referees will NOT be asked to assess your application. Please do **not** incorporate the names of your non-referees in this application form.



Netherlands Organisation for Scientific Research

**Vernieuwingsimpuls
Innovational Research Incentives Scheme
Grant application form (pre-proposal) 2018**

Ultra-resolution in optical nanoscopy

Vici scheme

Bernd Rieger

Please submit this signed application form to NWO in PDF format. For applications to all domains except ZonMw use the ISAAC system. For applications to ZonMw, use the ProjectNet system. Please do not use any security locks or bookmarks in the PDF file. For any technical questions regarding submission, please contact the ISAAC helpdesk (isaac.helpdesk@nwo.nl) or the ProjectNet helpdesk (projectnet@zonmw.nl), respectively.