1. Applicants and corresponding 3 recent and relevant publications:

Kristin Grußmayer

1. V. Navikas, A. C. Descloux, K.S. Grussmayer, S. Marion, A. Radenovic, Adaptive optics enables multimode 3D super-resolution microscopy via remote focusing. *Nanophotonics* June 2021, 10, 9, 2451-2458.
2. K. S. Grußmayer*, T. Lukes, T. Lasser, A. Radenovic*, Self-blinking Dyes unlock High-order and Multi-plane Super-resolution Optical Fluctuation Imaging, *ACS Nano* June 2020, 14, 7, 9156–9165.
3. K.S.Grußmayer*, S.Geissbuehler, A.Descloux, T.Lukes, M.Leutenegger, A.Radenovic, T. Lasser*, Spectral Cross-Cumulants for Multicolor Super-resolved SOFI Imaging, *Nat Commun* June 2020, 11, 3023.

Gijsje Koenderink

1. C. Alkemade, H. Wierenga, V.A. Volkov, M. Preciado López, A. Akhmanova, P.R. Ten Wolde, M. Dogterom, G.H. Koenderink. Cross-linkers at growing microtubule ends generate forces that drive actin transport. *Proc Natl Acad Sci U S A*. March 2022, 119(11):e2112799119
2. T. Xu, C. Langouras, M.A. Koudehi, B.E. Vos, N. Wang, G.H. Koenderink, X. Huang, D. Vavylonis, Automated Tracking of Biopolymer growth and Network Deformation with TSOAX, *Sci Rep.* Feb 2019, 8,9(1):1717.
3. Y. Mulla, F.C. MacKintosh, G.H, Koenderink. Origin of Slow Stress Relaxation in the Cytoskeleton. *Physical Review Letters* May 2019,122(21):218102

2. Title: Quantitative imaging of cytoskeletal crosstalk at the nanoscale in brain cancer

3. Abstract:

Glioblastoma is a terribly aggressive brain cancer that is currently untreatable. There is growing evidence that the lethal invasiveness associated with glioblastoma is due to defects in the interactions (crosstalk) between the three filament systems that together make up the cytoskeleton, namely actin, microtubules and intermediate filaments. The high density of cytoskeleton components prevents their characterization and analysis of interdependence using conventional, diffraction-limited microscopy. We wish to establish 3D multicolor super-resolution imaging at the nanoscale based on single-molecule imaging. This will enable segmentation and quantitative analysis of the three cytoskeleton networks in glioblastoma cell lines with different compositions of intermediate filaments. Establishing a collaboration between cell mechanics (GK) and super resolution imaging labs (KG) opens the perspective for joint development of correlative methods to connect the mechanoresponsiveness of single cells with high resolution imaging to unravel the molecular causes of cancer cell malignancy.

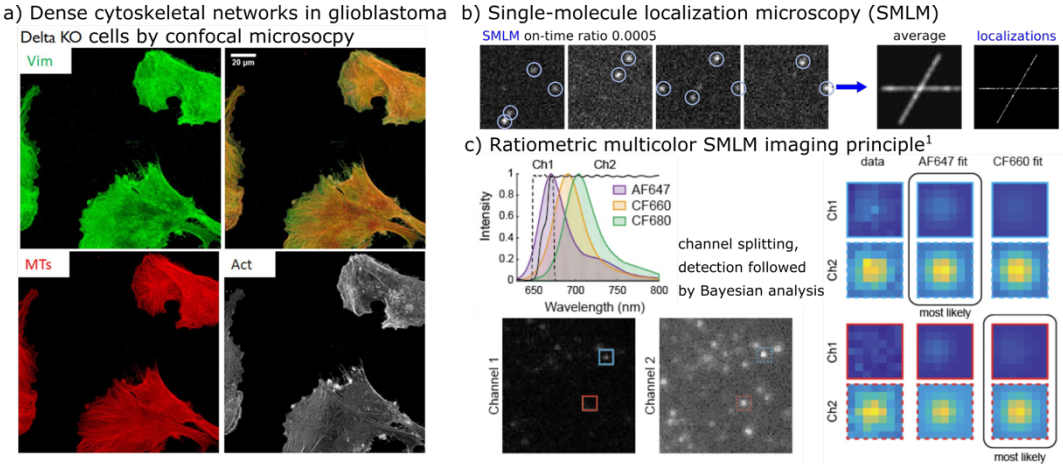
4. Benefit to the synergy of KIND:

We propose to combine the expertise of the Koenderink lab in (cancer) cell mechanics with the innovative nanoscale imaging developments of the Grußmayer lab to study the role of cytoskeletal crosstalk in brain cancer. Our collaboration will allow us to quantitatively investigate the co-localization and co-alignment of the cytoskeleton networks using super-resolution fluorescence microscopy to dissect the cytoskeletal crosstalk that underlies invasive migration of cancer cells. Moreover, this project will help the Grußmayer lab to move from a focus on imaging methodology development to addressing biologically relevant problems. Our ambition is that this synergy grant lays the basis for a long-term collaboration to further develop the imaging platform and correlate it with mechanical measurements. An exciting possibility would be to investigate the organization of the composite cytoskeletal networks in migrating cells on soft brain-mimicking substrates. This could be achieved by developing 3D multicolor self-blinking super-resolution optical fluctuation imaging combined with sensorless adaptive optics for aberration correction in this challenging imaging environment. With the results of this collaboration, we will apply for further funding (e.g., an NWO ENW-M2 or KWF grant) to investigate the physical mechanisms underlying the invasion of cancer cells in the brain.

6. Description of the proposed research:

Background: Cancer cells possess strikingly common mechanical features that are thought to facilitate aggressive tissue invasion**.** They are softer than their healthy counterparts and they can switch between different migration modes to effectively disseminate in tissues. Their unusual mechanics goes hand in hand with characteristic cell shapes, which are highly polarized and protrusive compared to healthy cells. The molecular mechanisms behind these pathological transitions are mostly unknown. Nevertheless, it is clear that the **cytoskeleton** is a key player. This structural scaffold of the cell is built of three distinct filament types that differ in their mechanical rigidity and assembly dynamics. The **actin** cytoskeleton is the engine behind cell migration because it generates the forces necessary to drive the cell forward[1]**. Microtubules** play a steering role by establishing front-rear cell polarity necessary for directional migration. **Intermediate filaments (IFs)** were traditionally considered merely a reinforcing network, but recent evidence indicates that they contribute to cell migration by regulating the architecture of the actin and microtubule cytoskeletons through physical and signaling interactions**.** However, the high density and nanoscale dimensions of these cytoskeletal networks in cancer cells hinder quantitative analysis of structural details and prevent direct observation of network interactions or co-assembly using conventional confocal microscopy.

The specific aim of this proposed collaboration is to develop fluorescence super resolution imaging and quantitative analysis of all the three cytoskeletal components to investigate cytoskeletal crosstalk in brain cancer cells.



Approach: State-of the-art **single molecule localization microscopy (SMLM)** achieves 5-20nm localization precision using far-red fluorescent dyes[2.] The high molecular density of the three cytoskeletal networks is a challenge for the precise localization of single blinking fluorophores. We will therefore set up multicolor super-resolution imaging based on a new **ratiometric imaging approach**[3], where we can use the best performing dyes (AF647, CF660C & CF680) to label the different filaments using immunofluorescence. By splitting the fluorescence into a high intensity, high resolution imaging channel and a classification channel that collects the low wavelength front of the emission spectrum, 3-4 spectrally close far-red dyes can be distinguished**.** This will enable multicolor imaging without the need to precisely co-register color channels and without increasing the molecular footprint, which is important for high density SMLM. We will analyze the cytoskeleton images using a dedicated algorithm for filamentous networks co-developed by Koenderink[4], which allows **quantification of filament lengths, orientations, and junction points to uncover the interaction of different cytoskeleton filaments.** As a biological model system, we will use gene-edited glioblastoma cells where we manipulate the molecular composition of the intermediate filament cytoskeleton. Recently, the Koenderink lab and collaborators found that glioblastoma cells presenting different isoforms of the intermediate filament protein **GFAP** strongly differ in overall cytoskeletal organization and cell invasiveness on glass and on soft brain-mimicking matrices.

References:

1. F. Huber, A. Boire, M. Preciado López, G.H. Koenderink, *Curr. Op. in Cell Biology* 2015, 32, 39-47
2. R. Diekmann, M. Kahnwald, A. Schoenit, J. Deschamps, U. Matti, J. Ries, *Nat Methods*. 2020;17(9):909-912.
3. Siemons ME, Jurriens D, Smith CS, Kapitein LC, *bioRxiv*, https://doi.org/10.1101/2022.01.14.476290
4. T. Xu, C. Langouras, M.A. Koudehi, B.E. Vos, N. Wang, G.H. Koenderink, X. Huang, D. Vavylonis, *Sci Rep.*2019, 8,9(1):1717.