

# ONi



Neuroscience

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# Nanoimager

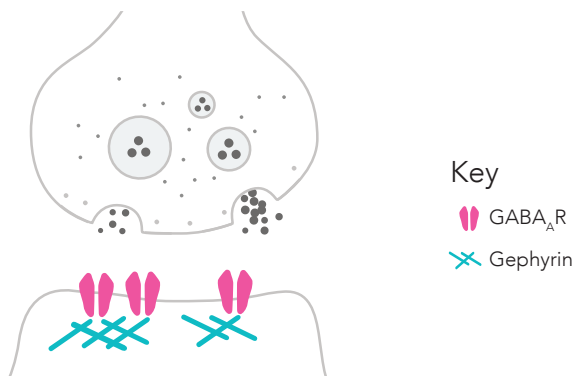
Single-molecule imaging in neuroscience



# Quantitative super-resolution reveals nanoscale recruitment of GABA<sub>A</sub> receptors following Bicuculline treatment

## Introduction

The function of the nervous system is to transmit information along the whole body. This transmission is driven by microscopic areas in neurons called synapses in which excitatory-inhibitory activity is balanced in order to achieve proper signal transmission. Inhibitory synapses are critical for controlling processing of information, cognition and behavior. Disruption of inhibitory synaptic function leads to alterations of behavior and contributes to numerous brain pathologies such as epilepsy and schizophrenia<sup>1</sup>. Inhibitory synapses are dynamic structures, capable of undergoing multiple forms of long-term plasticity, which alters the strength, related to neurotransmitter release, and the efficacy of synaptic inhibition. One of the major components of these synapses is the GABA<sub>A</sub> receptor (GABA<sub>A</sub>R). The number of GABA<sub>A</sub>Rs clustered in synapses determines the synaptic strength, along with its scaffold protein gephyrin (Schematic 1). Therefore, being able to measure the abundance of postsynaptic inhibitory proteins and how they change in response to synaptic plasticity, diseases and drug treatment at the single synapse level are important readouts in understanding neurological disorders associated with deficits in inhibitory neurotransmission. In this study, human neurons were treated with Bicuculline, a chemical compound that blocks the inhibitory action of GABA<sub>A</sub>Rs, to induce synaptic scaling of inhibitory synapses. As a consequence, neurons respond to elevated activity by modifying the receptor number in the postsynaptic region<sup>2</sup>.



## Challenge

Despite the numerous efforts to unveil the organization and stoichiometry of inhibitory synapses, quantitative measurements are still highly challenging. Molecular resolution is required to be able to visualize and quantify protein numbers in synapses. Single-molecule localization microscopy (SMLM), such as dSTORM (direct stochastic optical reconstruction microscopy), is able to overcome the diffraction limit and provide single-molecule sensitivity up to 20 nm resolution. This fluorescence-based technique offers an elegant

**Schematic 1** | Inhibitory synapse showing a presynaptic side where neurotransmitters are released through vesicles and a postsynaptic side with neuroreceptors, such as GABA<sub>A</sub>R, and scaffolding proteins, such as gephyrin.

## Summary

The Nanoimager is capable of performing multicolor super-resolution imaging in neuronal cultures. Among these techniques, dSTORM is commonly used to determine the molecular organization of protein complexes in synapses. With the Nanoimager it is possible to:

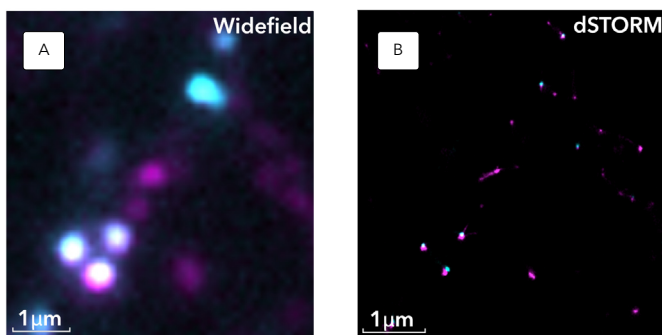
- Visualize synaptic protein organization with 20 nm resolution.
- Perform multicolor imaging of synaptic protein complexes, providing co-localization information of different targets at the nanometer scale.
- Determine quantitative subsynaptic parameters, such as the area, density and number of protein clusters per synapse or relative protein number per cluster.

solution to characterize nanoscale features in synaptic protein complexes. Through the multicolor capabilities of super-resolution imaging, it is now possible to disclose critical morphological information and apply detailed quantitation that lead to a better understanding of inhibitory synapse organization and how it reaches the subsynaptic domains. Inhibitory proteins such as GABA<sub>A</sub>Rs or gephyrin are organized in protein nanodomains along the synapse<sup>3</sup>. However, some quantitative measurements are still challenging even for super-resolution methods. Fortunately, dSTORM provides the possibility to extract stoichiometric features from the protein nanodomains. Each nanodomain will be represented by clusters of localizations coming from dSTORM blinking events. By comparing rendered images, it is possible to extract quantitative parameters in terms of the number of localizations per rendered cluster when structural changes occur, like in the case of neurons treated with Bicuculline with respect to control neurons.

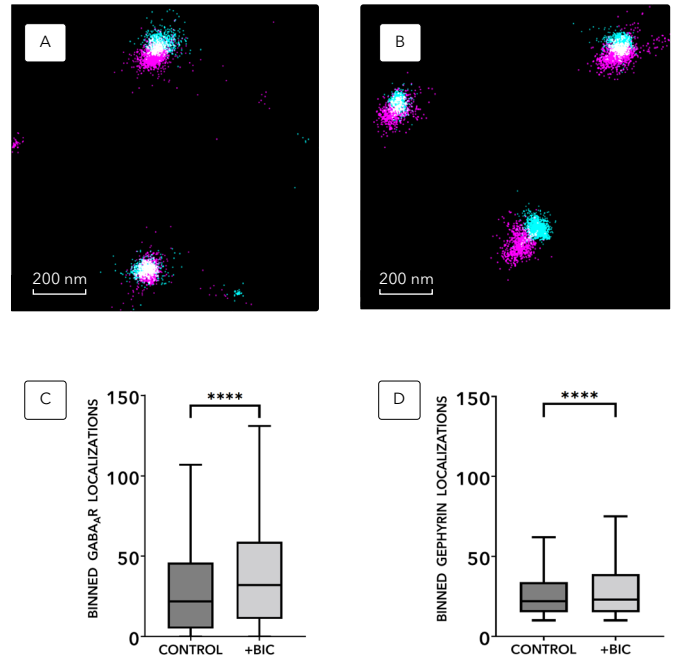
## Results

Healthy human iPSCs (induced pluripotent stem cells)-derived glutamatergic neurons were treated with 10 $\mu$ M Bicuculline for 24 hours prior to fixation. Fixed neurons were surface-stained for GABA $_A$ R gamma2 subunit, which is exclusively present in synaptic GABA $_A$ Rs, followed by permeabilization and staining for gephyrin. Primary antibodies targeting GABA $_A$ R were coupled with secondary antibodies labeled with Alexa Fluor<sup>®</sup> 647, whereas gephyrin was immunostained with antibodies conjugated with Alexa Fluor<sup>®</sup> 568. Dual color dSTORM was then performed on dendrites to visualize the synaptic structure at nanoscale resolution (Figure 1). The rendering of the localization maps of the distribution of GABA $_A$ Rs and gephyrin was performed during real-time acquisition in the Nanoimager software. The localization images were analyzed through the ONI online clustering software (CODI), which turned GABA $_A$ R and gephyrin localizations into clusters that could be quantified. Only clusters containing localizations of both proteins next to each other were considered, in order to discard extra-synaptic receptors from the analysis (Figures 2A, B). Following Bicuculline treatment, an increase in the number of localizations in GABA $_A$ R and gephyrin clusters was observed (Figures 2C, D), indicative of recruitment of GABA $_A$ R

and gephyrin to postsynaptic sites and increased inhibition within neurons. These findings demonstrate that dSTORM can be used to uncover interesting quantitative features underlying inhibitory synaptic plasticity.



**Figure 1** | Dual color imaging of GABA $_A$ R (magenta) and gephyrin (cyan). A) Widefield diffraction limited image. B) 2-color dSTORM reconstruction.



**Figure 2** | A, B) dSTORM imaging of synaptic boutons with GABA $_A$ R (magenta) and gephyrin (cyan) in control condition (A) and in neurons treated with Bicuculline for 24h (B). C, D) Tukey box plot of the number of localizations per cluster on both GABA $_A$ R (C) and gephyrin (D) for control and neurons treated with Bicuculline (+Bic). Whiskers represent the 1.5IQR. An unpaired T-test reveals a very significant difference in the case of the GABA $_A$ R and gephyrin clusters (\*\*\*\*  $P < 0.0001$ , control = 5063 synapses, +Bic = 7205 synapses).

## Solution with the Nanoimager

Optical microscopy is a very common technique used in Neuroscience to study signal transmission. However, several processes are happening at the molecular level, strongly limiting the research of neurological disorders. Therefore, nanometer resolution is essential to characterize the protein architecture of synapses. Traditionally, SMLM has been performed on complex and expensive systems, making them inaccessible and daunting for researchers to use. The Nanoimager has overcome these limitations as a benchtop-compatible microscope, capable of imaging and analyzing neuronal samples using SMLM in a

user-friendly manner. Its ease of use and the capability to render single-molecule localizations in real time allows a smooth workflow without the need for tedious training and specialized microscopy expertise. In addition, the Nanoimager comes with 4 laser lines that enable multi-channel imaging of spectrally separated dyes, and even simultaneous imaging of two targets. These features simplify the super-resolution experiments to study protein complexes in synapses and extract not only structural but also quantitative information of various targets, allowing neuroscientists to focus on the biological insights rather than the complexity of the technique.

## References

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2. Peng YR, Zeng SY, Song HL, Li MY, Yamada MK, Yu X. Postsynaptic Spiking Homeostatically Induces Cell-Autonomous Regulation of Inhibitory Inputs via Retrograde Signaling. *The Journal of Neuroscience*. 2010; 30 (16220-16231).
3. Crosby KC, Gookin SE, Garcia JD, Hahn KM, Dell'Acqua ML, Smith KR. Nanoscale Subsynaptic Domains Underlie the Organization of the Inhibitory Synapse. *Cell Reports*, 2019; 26 (3284-3297).

**ONI** Every  
Molecule  
Counts