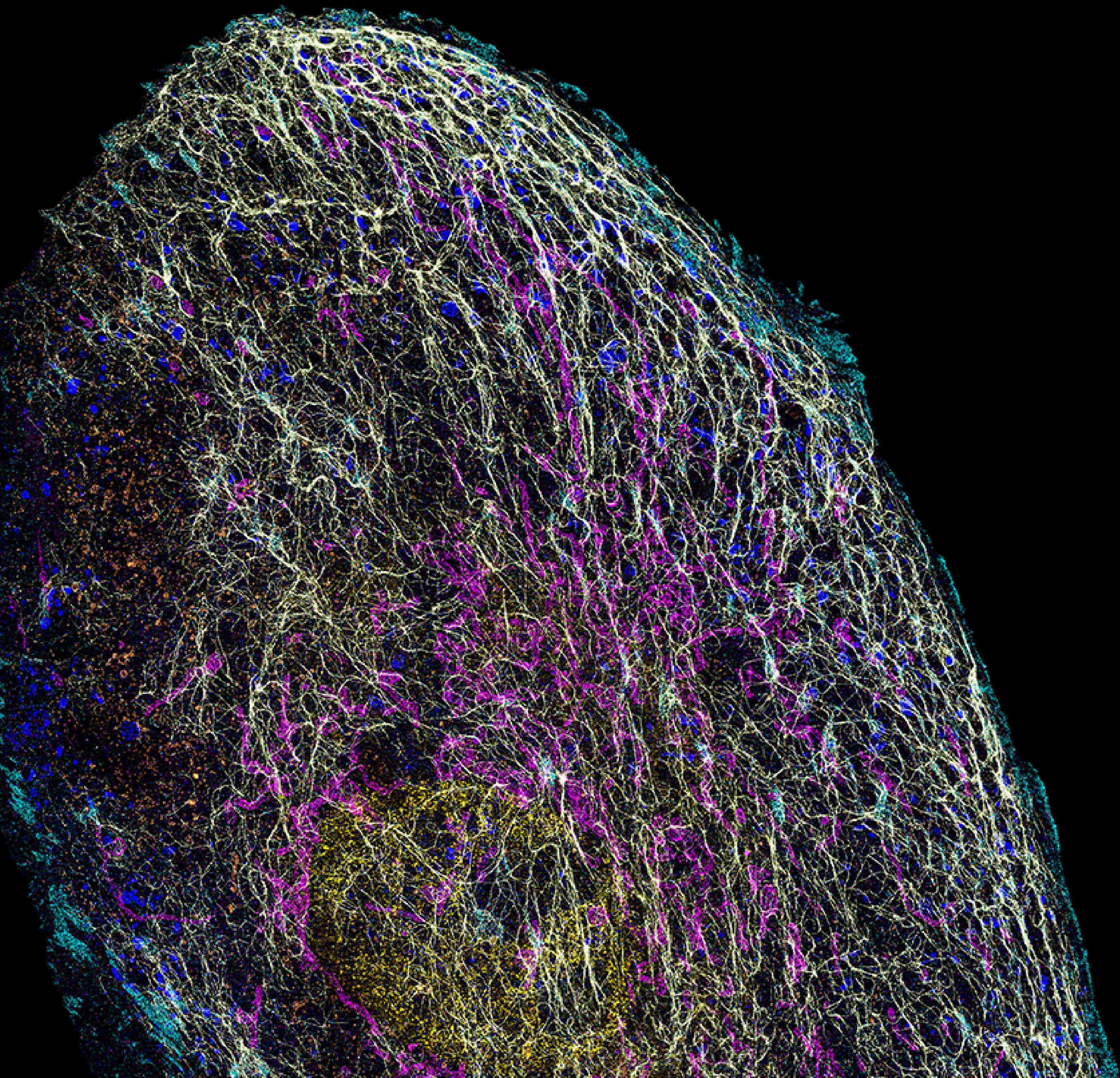
MASSIVE PHOTONICS

Beginner's Guide to DNA-PAINT



About us

Our Founders

Dr. Jürgen Schmied
CEO

Dr. Sebastian Strauß
CTO

Prof. Dr. Ralf Jungmann
CSO

Recognizing the growing demand for DNA-PAINT microscopy across the scientific community, Ralf, Jürgen, and Sebastian founded Massive Photonics in 2019 to make DNA-PAINT accessible to every scientist.

Our Mission

We empower scientists to solve critical research problems with DNA-PAINT technology. From high-performance labeling and imaging reagents to custom DNA–protein conjugates and collaborative research services, we deliver specialized tools and expertise to accelerate discovery.

Why Massive Photonics?

Born from the lab of DNA-PAINT pioneer Ralf Jungmann, Massive Photonics stands at the forefront of DNA-PAINT innovation. **We don't just follow the field - we help define it.** By combining cutting-edge products with expert-driven services, our team brings unmatched insight into every iteration of DNA-PAINT, from its origins to the latest breakthroughs. The result: powerful tools, trusted expertise, and the confidence to push your experiments further.

Contact us

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Understanding DNA-PAINT

What is DNA-PAINT microscopy?

DNA-PAINT (DNA-Point Accumulation for Imaging in Nanoscale Topography) is a super-resolution microscopy method that was pioneered in 2010 and first implemented in cells in 2014. It's part of the single-molecule localization microscopy (SMLM) family, and enables high spatial resolution, high multi-target imaging capabilities, and absolute target counting.

Why DNA-PAINT?

DNA-PAINT lets you visualize multiple targets in a single sample at nanoscale resolution, even in high-density environments. Fluorescent signal is never bleached during DNA-PAINT, enabling high target sampling and nanoscale resolution (< 20 nm).

Beyond visualization, DNA-PAINT delivers quantitative insight, enabling absolute target counting and spatial analysis. And with ready-to-use kits and seamless compatibility with existing analysis workflows and microscopes, getting started is straightforward.

How does DNA-PAINT work?

DNA-PAINT uses single stranded DNA (oligos) that transiently bind to their complements:

- **Imager strand** – a dye-labeled DNA oligo present in the imaging buffer
- **Docking strand** – a DNA oligo complementary to the imager strand that is delivered to the target of interest via affinity reagents (e.g. antibodies, single-domain antibodies)

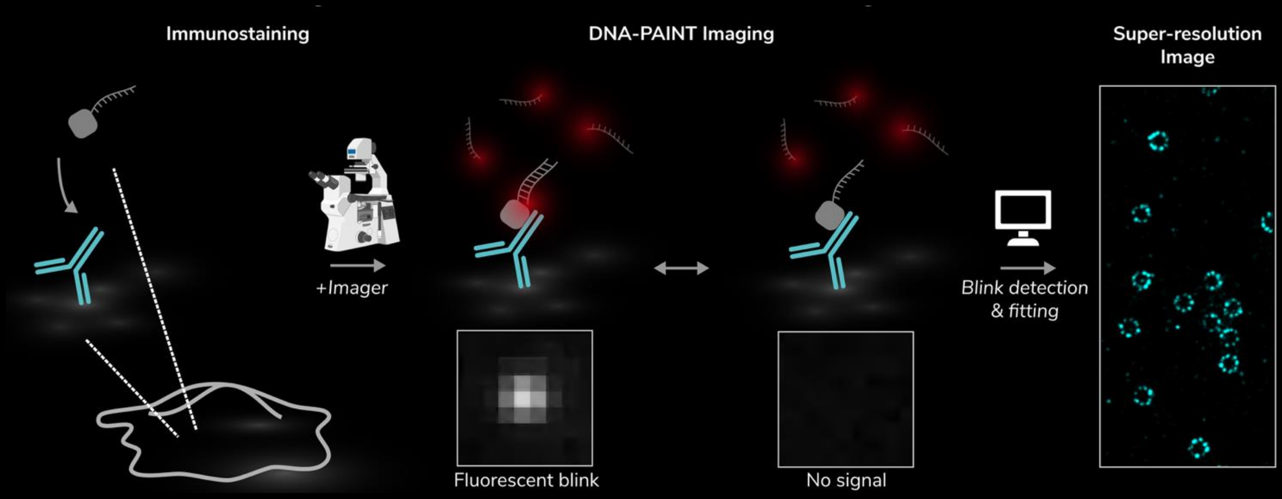

Figure 1: The DNA-PAINT concept. Cells are immunostained with a primary antibody and a secondary single-domain antibody (VHH) with a DNA-PAINT docking strand. After sample preparation, imaging buffer containing imager is added to the sample. During imaging, users see fluorescent blinks, which indicate an imager is transiently bound to the docking strand. Image acquired over minutes and blinks are detected and fitted in silico, creating the super-resolution image.

Imaging is performed in an aqueous buffer containing freely diffusing imager strands. When an imager transiently binds to its complementary docking strand, a fluorescent "blink" is generated. These diffraction-limited blinking events are captured over time and compiled into an image stack, which is then reconstructed *in silico* into a super-resolution DNA-PAINT image.

Because imagers are continuously replenished from solution, no bleaching occurs and blinking remains stable for hours, enabling dense target sampling and exceptional image quality.

How does DNA-PAINT compare to other super-resolution methods?

The best super-resolution microscopy method for your research questions depends on your project's specific imaging needs. DNA-PAINT stands out for researchers who require nanoscale resolution, (absolute) target counting, and want an easy method to image multiple targets within a single sample. See table below for an overview.

	DNA-PAINT	PALM	STORM	STED	SIM
Fluorescent emitter	Oligo with organic dye (imager strand)	PA-FPs	Organic dyes	Organic dyes, FPs	Organic dyes, FPs
Resolution	10 - 20 nm (1 nm RESI)	20 – 30 nm	20 – 30 nm	30 – 80 nm	60 – 100 nm
Multi-target imaging capabilities	8+ targets	1 – 2 targets	1-2 targets	2 – 4 targets	2 – 4 targets
Target counting capabilities	Strong	Moderate	Moderate	Limited	Limited
Target labeling strategy	Immunostaining	Genetic fusion of FP to target protein	Immunostaining	Immunostaining, FPs, Organic dyes	Immunostaining FPs, Organic dyes
Live-cell compatibility	Poor	Moderate	Poor	Limited	Moderate

Table 1: Overview of DNA-PAINT and other super-resolution microscopy methods. Parameter scores and values based on typical user experiences with widely accessible microscopes. Cutting-edge equipment or high expertise may influence results. PALM: Photoactivated Localization Microscopy, STORM: Stochastic Optical Reconstruction Microscopy, STED: Stimulated Emission Depletion, SIM: Structured illumination microscopy, FP: fluorescent protein, PA: Photo-activatable.

Reagents and Materials

What do I need for DNA-PAINT microscopy?

- **Affinity reagents conjugated to DNA-PAINT docking strands,** typically antibodies or single-domain antibodies (sdABs / VHHs)
- **Imager strands** which transiently bind to the docking strands to create a blinking signal
- **Imaging and Washing Buffer** compatible with DNA-PAINT imaging
- **A microscope** capable of single-molecule detection, such as a TIRF/HILO set-up with Hamamatsu ORCA-Fusion BT camera or similar (List below).
- **Data analysis software**, like the open-source software Picasso or other single-molecule localization microscopy (SMLM) tools

Our plug-and-play DNA-PAINT kits include antibodies or single-domain antibodies with DNA-PAINT docking strands, imager strands, imaging buffer, washing buffer, and antibody incubation buffer (blocking buffer).

How do I choose the right DNA-PAINT kit and sequences?

Our kits take the guess work out of sequence selection. See the decision tree below to determine which product type you need. Doing MINFLUX DNA-PAINT? Click here.

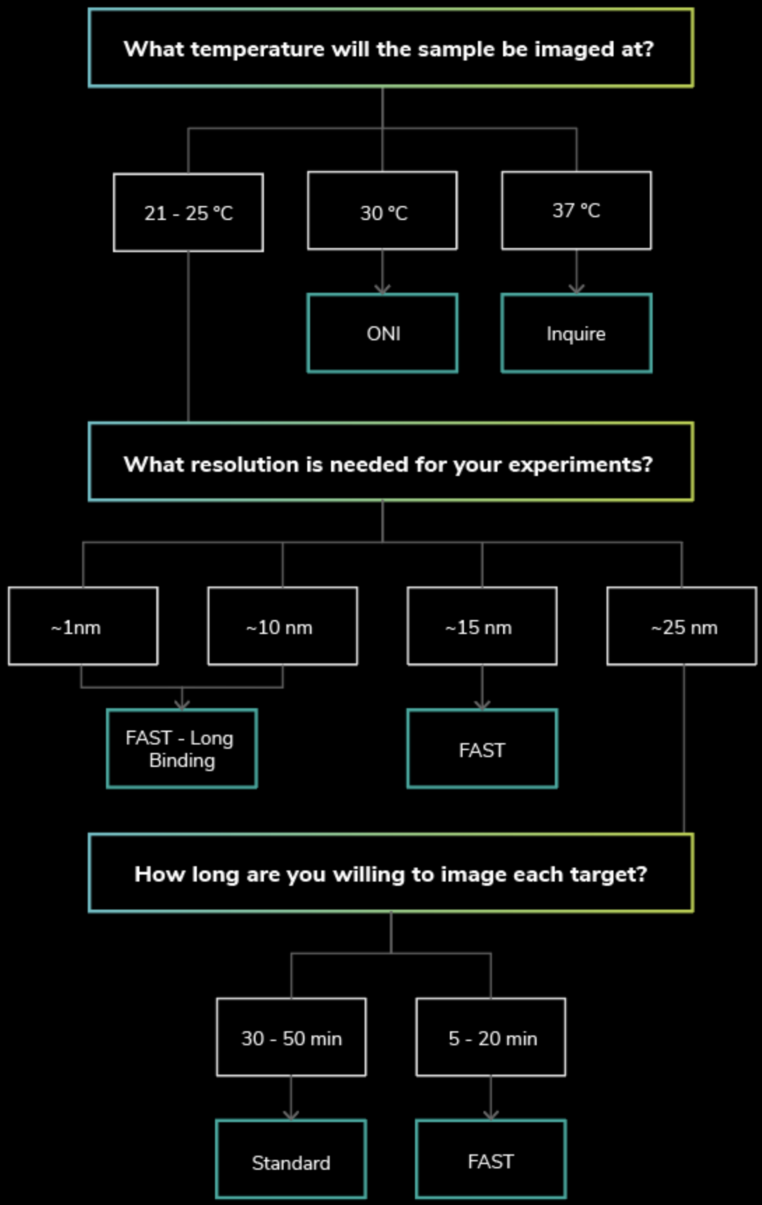

Figure 2: DNA-PAINT kit decision guide. Use the prompts to figure out which imager category works the best for your experiments. Match the imager type to the kit type using the text below.

Standard imagers in DNA-PAINT kits. FAST and FAST- Long Binding imagers in FAST-DNA-PAINT Kits and Multiplexing Kits. ONI imagers sold separately and in ONI kits. For 1 nm resolution, RESI kit required. For imaging in Epifluorescence or widefield illumination, fluorogenic imagers required.

Prefer a specific docking strand and imager sequence? No problem. We offer custom products with any sequence of your choosing.

What microscopes can I use for DNA-PAINT imaging?

DNA-PAINT imaging is typically performed with commercial single-molecule localization microscopy microscopes or home-built TIRF set-ups, which are common in Imaging Facilities, universities, and companies. The microscope should be able to detect single molecules and have sufficient laser power to observe blinking with a high singal-to-noise ratio.

DNA-PAINT imaging can be done on various commercial microscopes, including:

- Abbelight SAFe MN360
- abberior MINFLUX*
- Bruker Vutara VXL*
- Nikon CSU-W1 SoRA with Optical Photon Reassignment
- Nikon N-STORM
- ONI Nanoimager*
- ONI Aplo Scope*
- Zeiss Elyra

*See special requirements for this microscope below:

Microscope	Special requirement	Compatible Products
abberior MINFLUX	Imager strands with MINFLUX blinking kinetics	DNA-PAINT Kits FAST-DNA-PAINT Kits, must select FAST-LB imager
Bruker Vutara VXL	Imager strands compatible with Epifluorescence imaging	FAST-DNA-PAINT Kits, must select imager Cy3B Fluorogenic
ONI Nanoimager **ONI Aplo Scope**	Imager strands for imaging at higher temperatures	ONI DNA-PAINT Kits FAST-DNA-PAINT Kits, must inform Sales Team you need ONI imager

Table 2: Commercially available microscopes with special requirements for DNA-PAINT imaging.

Note this list of microscopes is not comprehensive. Feel free to contact us if you're unsure your microscope can perform DNA-PAINT.

Do I need to add an oxygen scavenging system to Massive Photonics Imaging Buffer?

Our Imaging Buffer delivers nanoscale resolution without the need for an oxygen scavenging system (OSS), but incorporating an OSS can enhance resolution and maintain performance when hours of imaging are required. For guidance on selecting the optimal system for specific imager dyes, we recommend the publication by Steen et. al.

Do you offer custom products?

Yes, customization is at the core of what we do. We offer custom conjugation of antibodies and single-domain antibodies (VHHs) from our kits with custom oligos (e.g., docking strands), as well as user-defined primary antibodies or proteins following a feasibility assessment.

We also provide custom imagers across a broad range of dyes to match your experimental needs. To discuss, inquire at info@massive-photonics.com.

Can I do MINFLUX with Massive Photonics DNA-PAINT kits?

Yes, many users successfully perform MINFLUX DNA-PAINT with our products. Compatible kits include DNA-PAINT Kits and FAST-DNA-PAINT Kits when FAST-LB imagers are used.

DNA-PAINT Imaging

What imager dyes work best for DNA-PAINT microscopy?

Cy3B is the highest-performing dye for DNA-PAINT microscopy, delivering higher spatial resolution, signal-to-noise ratios, and lower background than all other dyes.

For imaging in the red channel, ATTO 655 is the best choice. While the resolution is slightly lower than that of Cy3B, it offers high performance, specificity, and affordability.

Massive Photonics kits offer a selection between Cy3B or ATTO 655. Custom imager dyes available upon request.

How do I image my sample?

1. **Prepare your sample:** Follow your usual immunostaining protocol but replace your dye-labeled binder (antibody, VHH, etc.) with one conjugated to a DNA-PAINT docking strand, like those found in our kits.
2. **Add imagers:** Dilute imager strands in Imaging Buffer, apply to sample, place sample on the microscope. Find cells using the bright field or fluorescent signal. Check sample for single-molecule blinking. Imager concentration can be adjusted on the microscope as needed by diluting the imaging buffer on the sample or replacing the imaging buffer with one containing a higher imager concentration.
3. **Setting the imaging plane:** Adjust the focal plane and the illumination angle until single-molecule blinking appears in focus with the highest possible signal-to-noise ratio (SNR). If the target is tagged with a fluorescent protein, it can be used to find the focal plane. We recommend screening optimal blinking by starting at TIRF and slowly adjusting the illumination angle towards EPI. Cellular targets are typically imaged using TIRF to HILO illumination. EPI illumination is possible if using fluorogenic imagers (available in FAST-DNA-PAINT Kits).

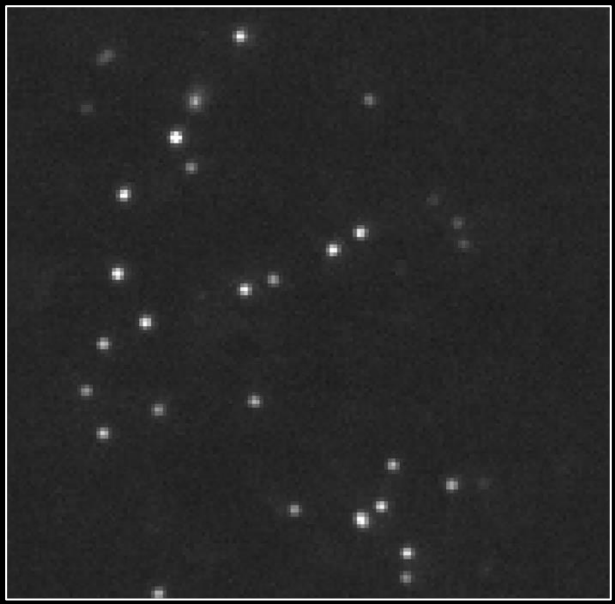

Figure 3: Example of a good DNA-PAINT imaging plane. Observed blinking has high signal-to-noise ratio and majority of blinks appear in-focus without overlapping. Image is a single frame taken from raw data of nuclear pore imaging. Sample prepared using Massive-TAG-Q-FAST Anti-GFP kit.

4. **Selecting imaging settings:** Please set according to our guidelines for the kit type, found below.

	DNA-PAINT Kits	FAST DNA-PAINT Kits Imager: FAST	FAST DNA-PAINT Kits Imager: FAST-LB
Laser Power*	~200 W/cm^2 (561 nm) ~300 W/cm^2 (640 nm)	~300 W/cm^2 (561 nm) ~500 W/cm^2 (640 nm)	~200 W/cm^2 (561 nm) ~300 W/cm^2 (640 nm)
Camera Integration Time	100 – 150 ms	30 – 75 ms	100 – 150 ms
Imaging time per target	20 – 50 min	5 – 20 min	15 – 30 min
Typical Imager concentration	1 – 5 nM	50 – 1000 pM	50 – 750 pM
Resolution	20 nm	15 – 25 nm Higher exposure time yields higher resolution	10 nm

Table 3: Recommended Imaging Settings for Massive Photonics DNA-PAINT kit categories.

*Laser power density highly depends on the microscope and illumination mode and should be optimized so a good signal-to-noise ratio is observed.

5. **Acquiring the image**: Typical experiments require 5 – 20 K camera frames, depending on imager concentration, target density, and overall sample preparation.

How do I image more than one cellular target in the same sample? (Exchange-PAINT)

When imaging more than one target in a single sample, targets must be labeled with orthogonal docking strands.

In most cases, multi-target imaging is performed using Exchange-PAINT (Figure 4), in which targets are imaged sequentially. Here's how it works:

1. Add Imaging Buffer 1, containing Imager 1
2. Image Target 1
3. Wash sample to remove Imaging Buffer 1
4. Add Imaging Buffer 2, containing Imager 2
5. Image Target 2
6. Wash sample to remove Imaging Buffer 2
7. Repeat for n targets

Washing can be performed manually using pipettes or with a microfluidics system. Washing the sample quickly three times with Massive Photonics Washing Buffer is typically sufficient to remove imaging buffer. We recommend practicing buffer exchange at your lab bench before trying it on your microscope. Don't worry – it only takes a few practice rounds to master. Fiducial markers, such as gold nanoparticles, can be used to correct drift occurring between imaging rounds and align post-processed images.

Not sure if all imager is gone? Check for residual blinking using your microscope before adding the next imaging buffer.

Want to reduce washing rounds? Use imagers that are labeled with spectrally distinct dyes, ex: Cy3B and ATTO 655. Instead of washing between imaging rounds, simply change lasers. Remember to use gold fiducials to correct for chromatic aberrations.

Want the highest possible resolution? Use Cy3b to image all targets.

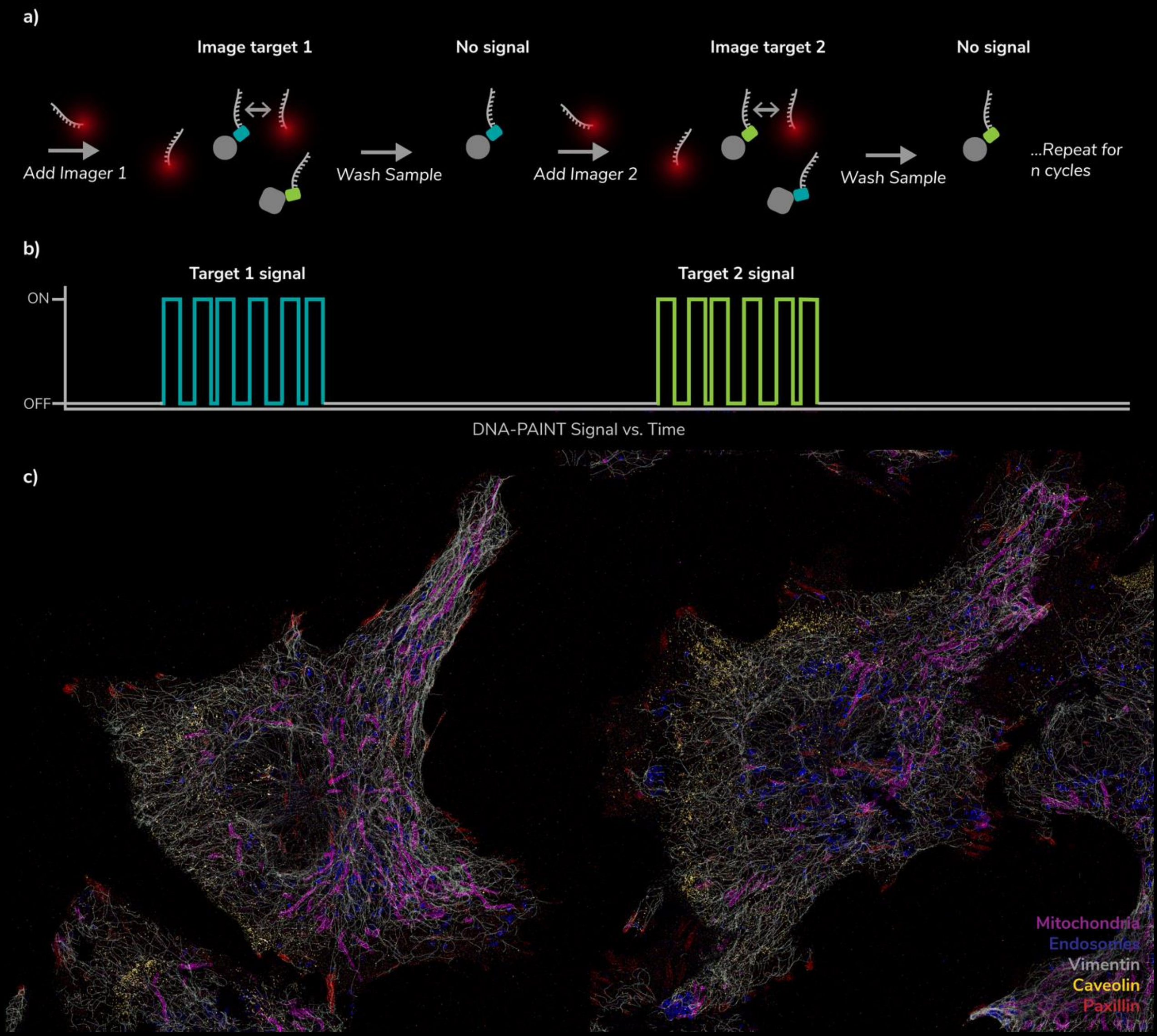

Figure 4: Multi-target imaging via Exchange-PAINT. (a) Schematic of Exchange-PAINT (b) Schematic of DNA-PAINT signal captured by the microscope. Signal peaks represent one imager-docking strand binding event, seen as a blink in the camera. (c) Example 5-target image acquired using Exchange-PAINT. Massive Photonics 5-Plex Multiplexing Kit used for sample preparation.

How do I count the number of target molecules in my sample?

Quantitative DNA-PAINT (qPAINT) allows you to count the exact number of molecules in your sample. qPAINT works by taking advantage of the predictable way imager strands bind to docking strands. Each time an imager binds, it creates a fluorescent "blink." The key insight: more targets = more frequent blinking. For example, an area with three targets will produce blinking events about three times more often than an area with just one.

To understand this note:

- **Bright time** (τ_b) – duration an imager stays bound to the docking strand
- **Dark time** (τ_b) – duration no imager is bound to docking strand

qPAINT focuses on the dark time between binding events. By measuring how often blinking occurs in a given area, it can determine how many binding sites, and therefore how many target molecules, are present.

To ensure accurate counting, a reference structure with a known number of docking strands is included. This creates a "kinetic fingerprint" that allows you to translate blinking frequency into absolute molecule numbers. Single proteins such as monomeric receptors or DNA-Origami structures can be used.

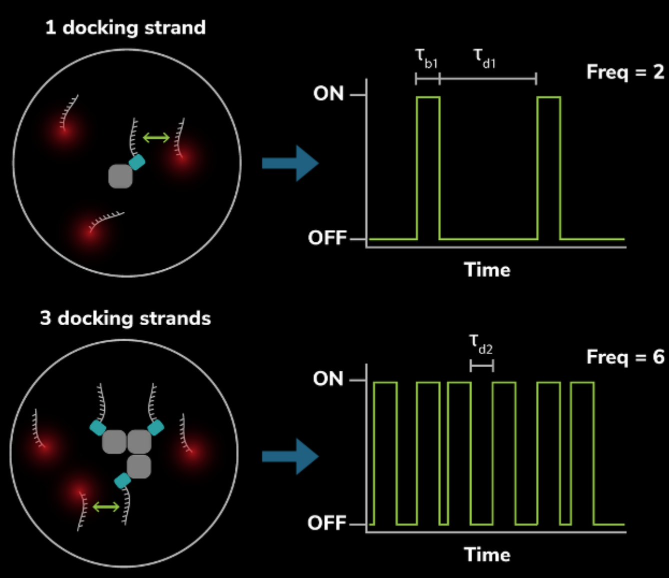

Figure 5: Quantitative DNA-PAINT. qPAINT schematic showing how number of docking strands, which represent target molecules, influence the binding frequency of imager strands within an area. Green signal peaks in DNA-PAINT signal vs. time plots represent one imager-docking strand binding event, seen as a blink in the camera. If three docking strands are present, binding frequency will be three times more often than if one docking strand is present. These kinetics can be used to calculate absolute target numbers. τ_b, bright time. τ_d, dark time. ON, imager bound. OFF, imager not bound. Freq, frequency.

See paper listed in the "Seminal DNA-PAINT Literature" section to learn more. We recommend gaining a thorough understanding of DNA-PAINT imaging before trying qPAINT.

How long does it take to image one target using DNA-PAINT?

Imaging duration depends on several factors including the DNA-PAINT sequence design, imager concentration, and target density. See Table 3 for more info.

Image Processing

How do I create a super-resolution image from my raw data?

Commercially available microscopes typically have built-in software for analyzing raw data from single-molecule localization microscopy methods like DNA-PAINT and offer analysis support.

We use the open-source program Picasso, created by the Jungmann lab, that was specifically developed for DNA-PAINT imaging. Picasso is consistently maintained and improved and has extensive documentation. Please see Picasso's readthedocs for the most up-to-date information. Paper 5 in the Seminal DNA-PANT Literature section explains Picasso thoroughly but doesn't cover functions added after 2017.

How do I align multi-target images after Exchange-PAINT?

When imaging multiple targets in the same sample, use fiducial markers (e.g., gold nanoparticles) for channel alignment.

If using Picasso: Open all drift corrected files in Picasso Render. Select the fiducial markers using the "Pick" tool in the "Tools" menu, then click "Align channels" in the "Postprocess" menu.

What's the best method for drift correction?

For drift correction, we highly recommend using fiducial markers such as gold nanoparticles and or a state-of-the-art drift correction algorithm such as AIM to enable true nanoscale resolution. We've found that using RCC-based drift correction alone, often the default in commercial microscopes, may reduce the effective resolution of the final image.

Final Thoughts

DNA-PAINT brings together resolution, multiplexing, and quantification to unlock a deeper understanding of biological systems. Throughout this guide, you've seen how its underlying principles translate into practical workflows, reliable results, and new experimental possibilities.

Whether you're just getting started or refining your approach, DNA-PAINT offers the flexibility and precision to grow with your research. At Massive Photonics, we're committed to making that journey as seamless as possible by providing the tools, expertise, and support to help you succeed at every step.

Need further support or have questions? Get in touch with our team.

Seminal DNA-PAINT Literature

1. **DNA-PAINT introduced:**
   Jungmann, R., Steinhauer, C., et al. Single-Molecule Kinetics and Super-Resolution Microscopy by Fluorescence Imaging of Transient Binding on DNA Origami. Nano Lett. 10, 11, 4756–4761 (2010). https://doi.org/10.1021/nl103427w
2. **Exchange-PAINT introduced**:
   Jungmann, R., Avendaño, M., Woehrstein, J. et al. Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. Nat Methods 11, 313–318 (2014). https://doi.org/10.1038/nmeth.2835
3. **Quantitative DNA-PAINT introduced**:
   Jungmann, R., Avendaño, M., Dai, M. et al. Quantitative super-resolution imaging with qPAINT. Nat Methods 13, 439–442 (2016). https://doi.org/10.1038/nmeth.3804
4. **RESI, a DNA-PAINT method for >1 nm resolution introduced**:
   Reinhardt, S.C.M., Masullo, L.A., Baudrexel, I. et al. Ångström-resolution fluorescence microscopy. Nature 617, 711–716 (2023). https://doi.org/10.1038/s41586-023-05925-9
5. **DNA-PAINT Microscopy Guide with step-by-step protocols**:
   Schnitzbauer, J., Strauss, M., Schlichthaerle, T. et al. Super-resolution microscopy with DNA-PAINT. Nat Protocols 12, 1198–1228 (2017). https://doi.org/10.1038/nprot.2017.024