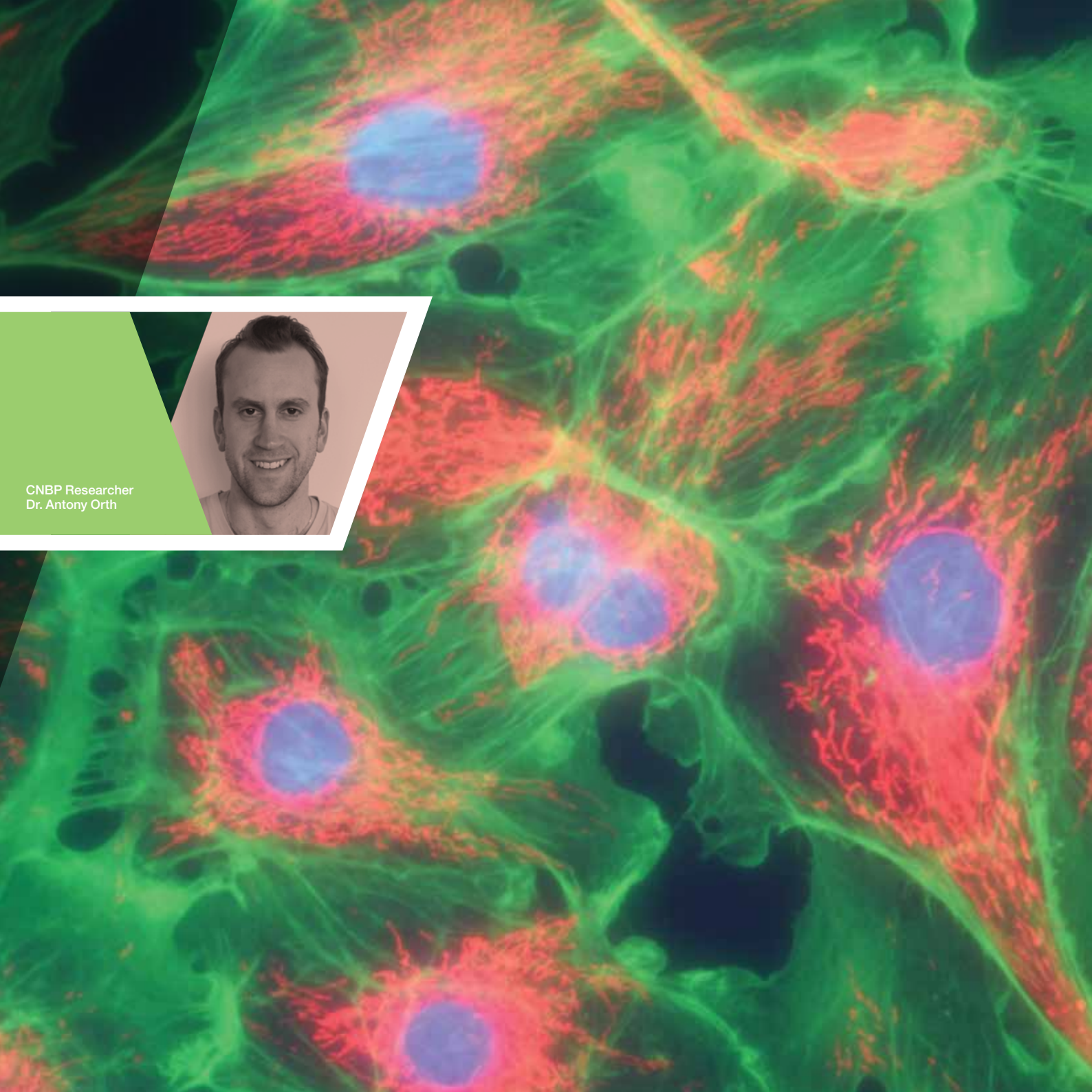

CNBP Researcher
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Case Study

Innovative Microscopy Techniques

IMPROVED MULTIPLEXING

Dr. Antony Orth

Fluorescence microscopy is one of the most widely used techniques in biology. Light emitting molecules called fluorophores tag specific targets in cells, each with a unique and identifiable colour.

Colour filters in the microscope can then select emission from any one of the types of fluorophores while blocking light originating from all others, resulting in information-rich images.

This approach is versatile, but there is a major limitation. The visible spectrum, where most fluorophores operate, can get crowded. The visible colour spectrum spans the range from 400nm to 700nm and only about 200nm of this range is available for fluorescence emission.

A typical fluorophore emits over a 50nm range of the colour spectrum. For colour filtering to work well, the fluorescent emission from different species should not overlap—in other words they should have distinct enough colours.

However, in dividing up 200nm of the visible spectrum into 50nm segments, the colours of fluorescent emitters blend together when you attempt to squeeze in more than four colours. In order to highlight more targets for more highly multiplexed experiments, there is a need to use another property to differentiate between fluorescent species.

At the CNBP, we have developed a technique called “bleaching-assisted multichannel microscopy” (BAMM) to increase multiplexing in fluorescence microscopy.

In leveraging photo-bleaching for increased multiplexing, the CNBP has turned what has historically been considered a detrimental effect into an extremely useful phenomenon.

Instead of using colour to differentiate between fluorophores, we use the 4th dimension of time and exploit a phenomenon called photo-bleaching—the dimming of a collection of fluorophores or pigments under repeated exposure to light.

Because each type of fluorophore photo-bleaches at a different rate, we can differentiate between fluorophores without using any colour information. When paired with colour information, this added dimension of contrast enables scientists to use 2-3 times more types of fluorescent molecules, all in a single sample.

Current approaches to increased multiplexing involve significantly expensive hardware. In contrast, BAMM actually simplifies microscope design by obviating the need for colour filters in some cases.

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