PRACTICE LESSON…

…To be ready for the Lab!

Tips on how to…

1

FIX THE OOCYTES
(oocytes fixation methods according to nuclear staining)

2

MOUNT THE OOCYTES ON A SLIDE

3

STAIN THE OOCYTES

4

RECOGNIZE THE PROPER OOCYTE NUCLEAR STAGE ON THE MICROSCOPE
(with different types of dyes)

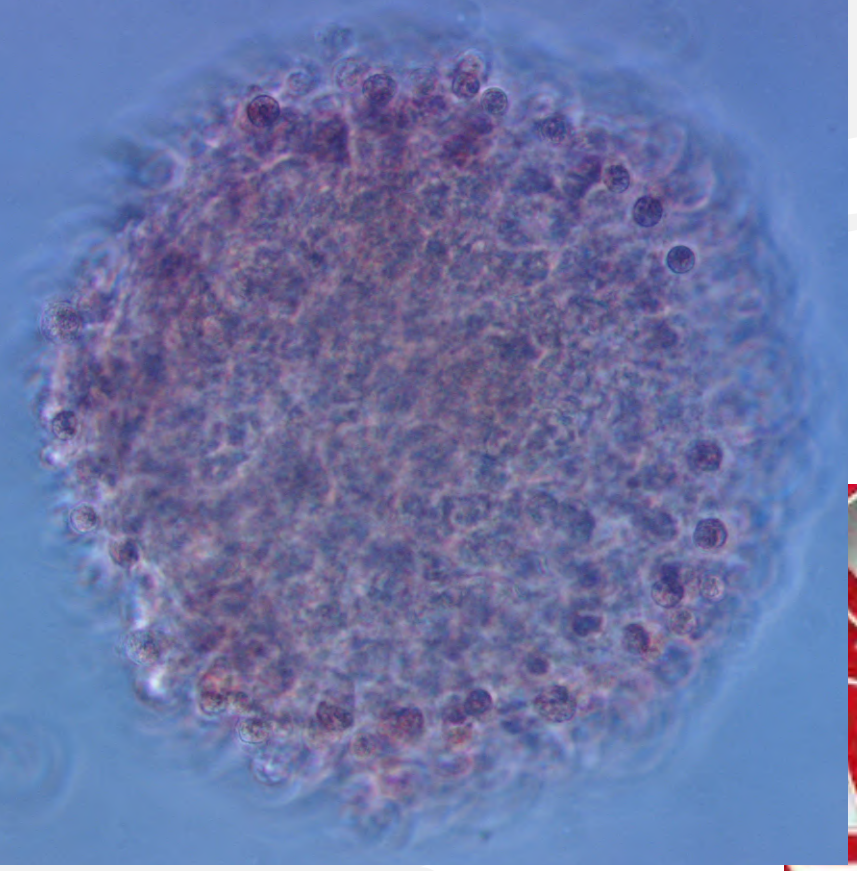
Why evaluate the oocyte nuclear stage?

- **For IVF Lab procedures**: The oocyte is considered mature (from a nuclear point of view), and therefore usable for in vitro insemination, if it has reached the metaphase II stage.
- **For research purposes**: the meiotic competence needs to be evaluated to assess proper cultural method protocols or specific treatments.

What types of dyes can we use to evaluate the nuclear stage of oocytes?

- **Hoechst**: is a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA. It may be used on live or fixed cells, and are often used as a substitute for another nucleic acid stain, DAPI. The key difference between them is that the additional ethyl group of Hoechst 33342 makes it more lipophilic, and thus more readily to cross intact cell membranes

- **Lacmoid:** phase contrast dye specific for chromatin. The substantial difference with the first dyes is that with Lacmoid we can see not only the nuclear stage, but also the quality of the cytoplasm



PAY ATTENTION!

Before oocytes fixation…

THE OOCYTES NEED TO BE DENUDED FROM CCs!

LACMOID STAINING

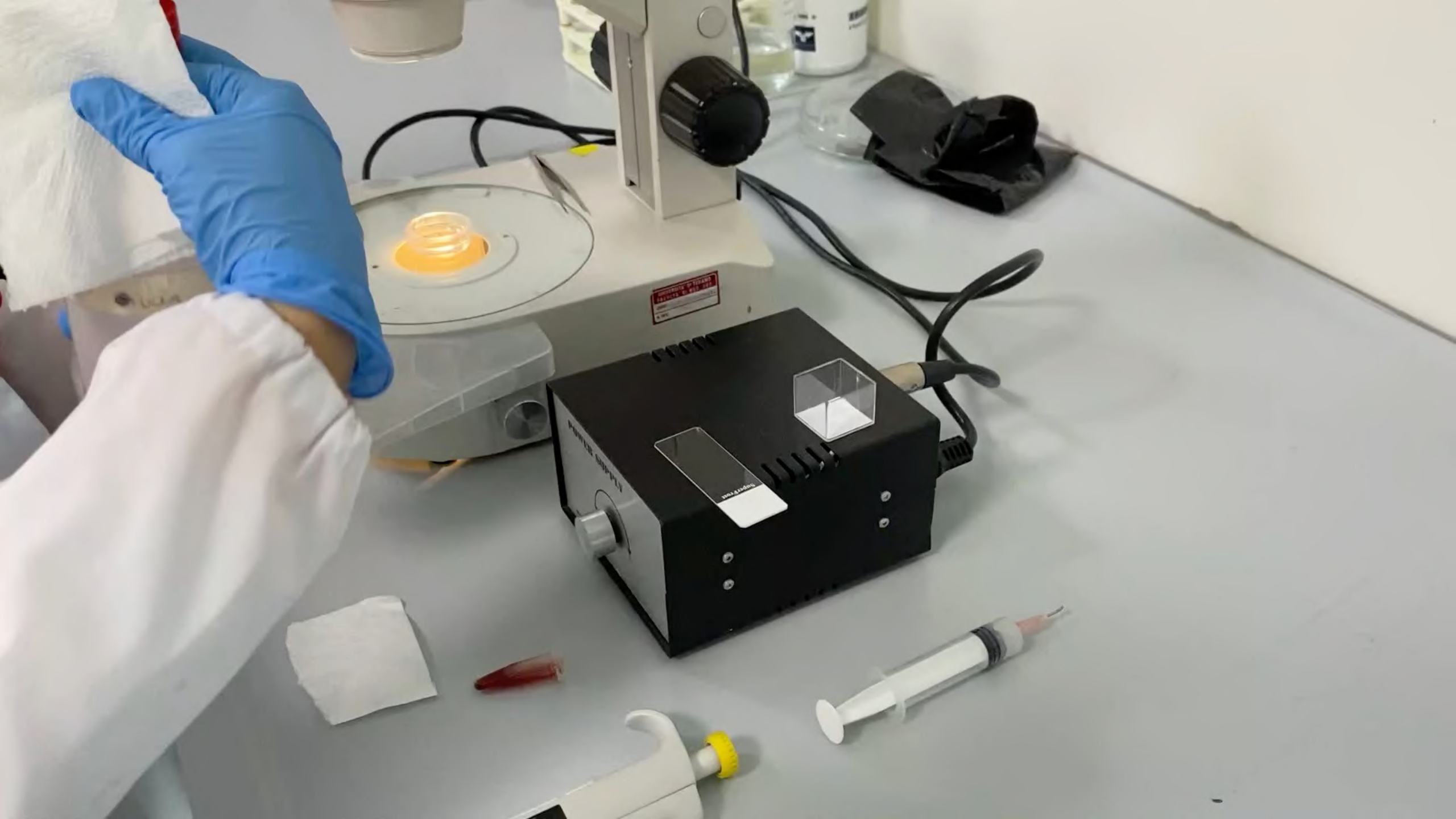
- ✓ Mount the oocytes on a slide:
  1. Clean the slide with ethanol 70%; then, make 2 parallel vaseline strips on the horizontal borders (to avoid the oocytes overpressure by the cover slide).
  2. Take the oocytes from the petri dish with DPBS and release the drop in the middle of the slide and cover it with cover slide.
  3. Apply little pressure with a needle or forcep on the border of the cover slide until the formation of a «bubble». After that, verify the correct mounting of the oocytes by injecting some liquid through the cover slide (the oocytes do not have to move!)

- ✓ Move the slide with the oocytes in a rack with acetic acid and ethanol (ratio 1:3) and leave the oocytes overnight.

- ✓ Move the slide with the oocytes in another rack with ethanol for 10 minutes. Meanwhile, prepare the lacmoid dye: take 750 µL distilled water and add 250 µL Lacmoid stock solution. Centrifuge at maximum speed (rpm) for 10 minutes.

- ✓ Take 50 µL of Lacmoid dye solution already prepared and release it near the cover slide, using a piece of paper towel on the opposite slide: in this way, the stain solution can pass through the cover slide for capillarity. NB: if the oocytes should move, apply again a little pressure on the cover slide to be sure they are well mounted.

(to clarify, see the video on the next slide)

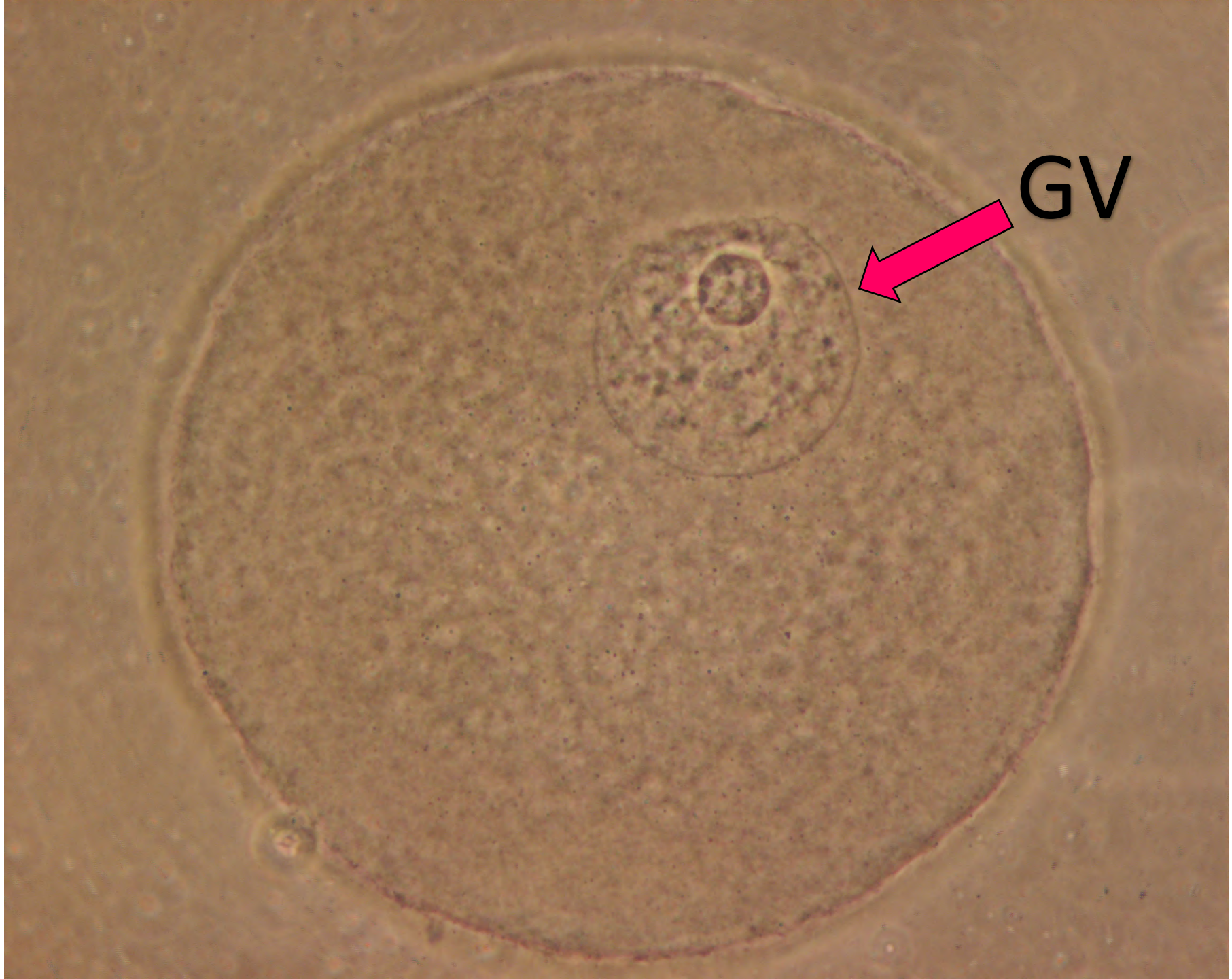


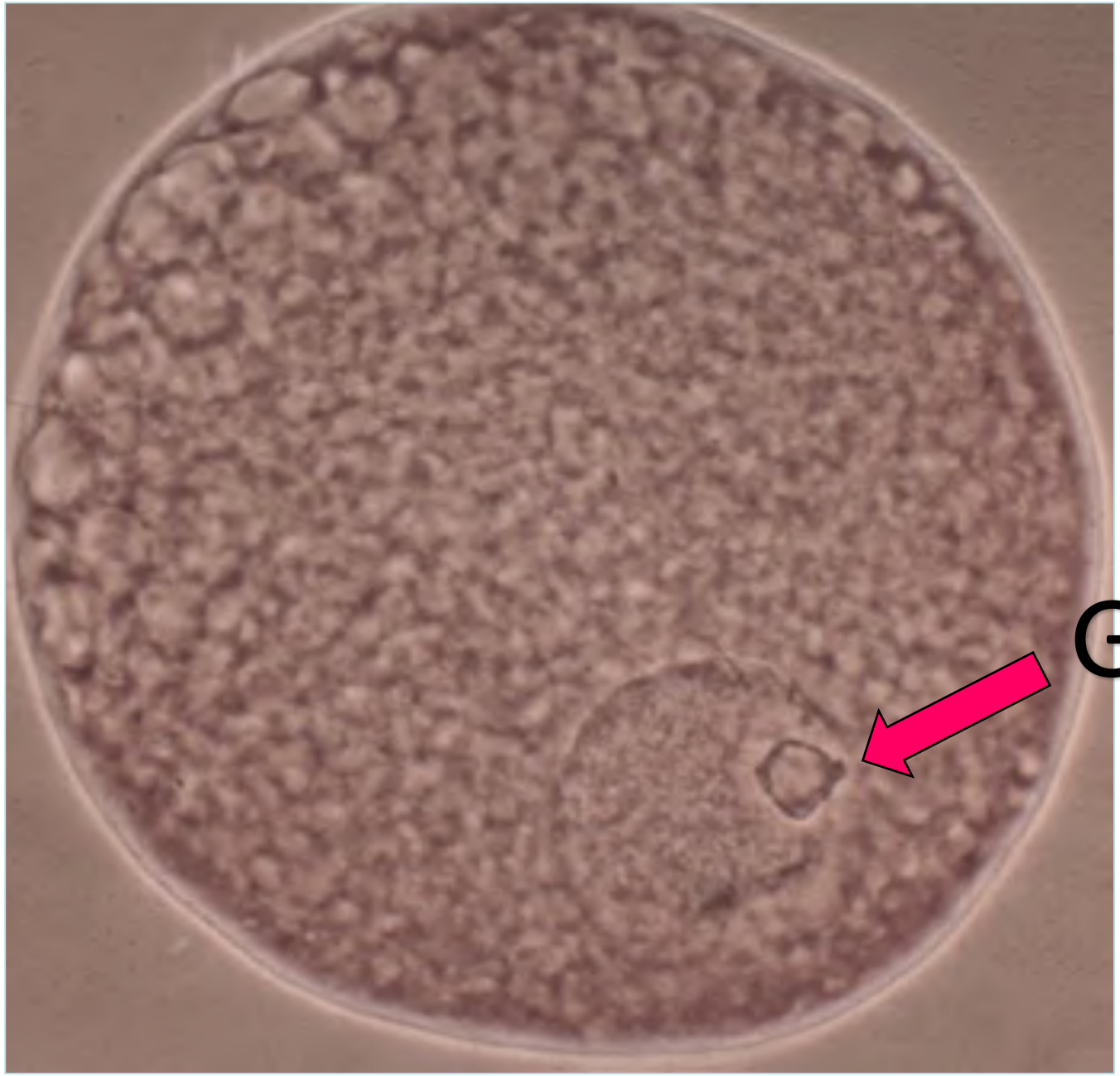
HOECHST 33342 STAINING

- ✓ Before the staining, fix the oocytes in paraformaldehyde 4% for 30 minutes. Cover the oocytes's petri with tinfoil to protect the oocytes from light and wait 30 minutes. Then, wash the oocytes in Dulbecco's PBS (with Ca2+ and Mg2+) thrice for 10 minutes each time.

- ✓ Move the oocytes in a petri dish (35 mm) with Dulbecco's PBS (with Ca2+ and Mg2+). Release into the petri dish tot volume (µL) of Hoechst stain solution according to the petri volume (ratio 1:1000. For example: 2 µL Hoechst in 2000 µL Dulbecco's PBS). Cover the oocytes's petri with tinfoil and wait 30 minutes.

- ✓ Mount the oocytes on a slide:
  1. Clean the slide with ethanol 70%; then, make 2 parallel vaseline strips on the horizontal borders (to avoid the oocytes overpressure by the cover slide).
  2. Take the oocytes from the petri dish and release the drop in the middle of the slide and cover it with cover slide.
  3. Apply little pressure with a needle or forcep on the border of the cover slide until the formation of a «bubble». After that, verify the correct mounting of the oocytes by injecting some liquid through the cover slide (the oocytes do not have to move!)
  4. Evaluate the nuclear stage of the oocytes using a fluorescent microscope (UV).


GV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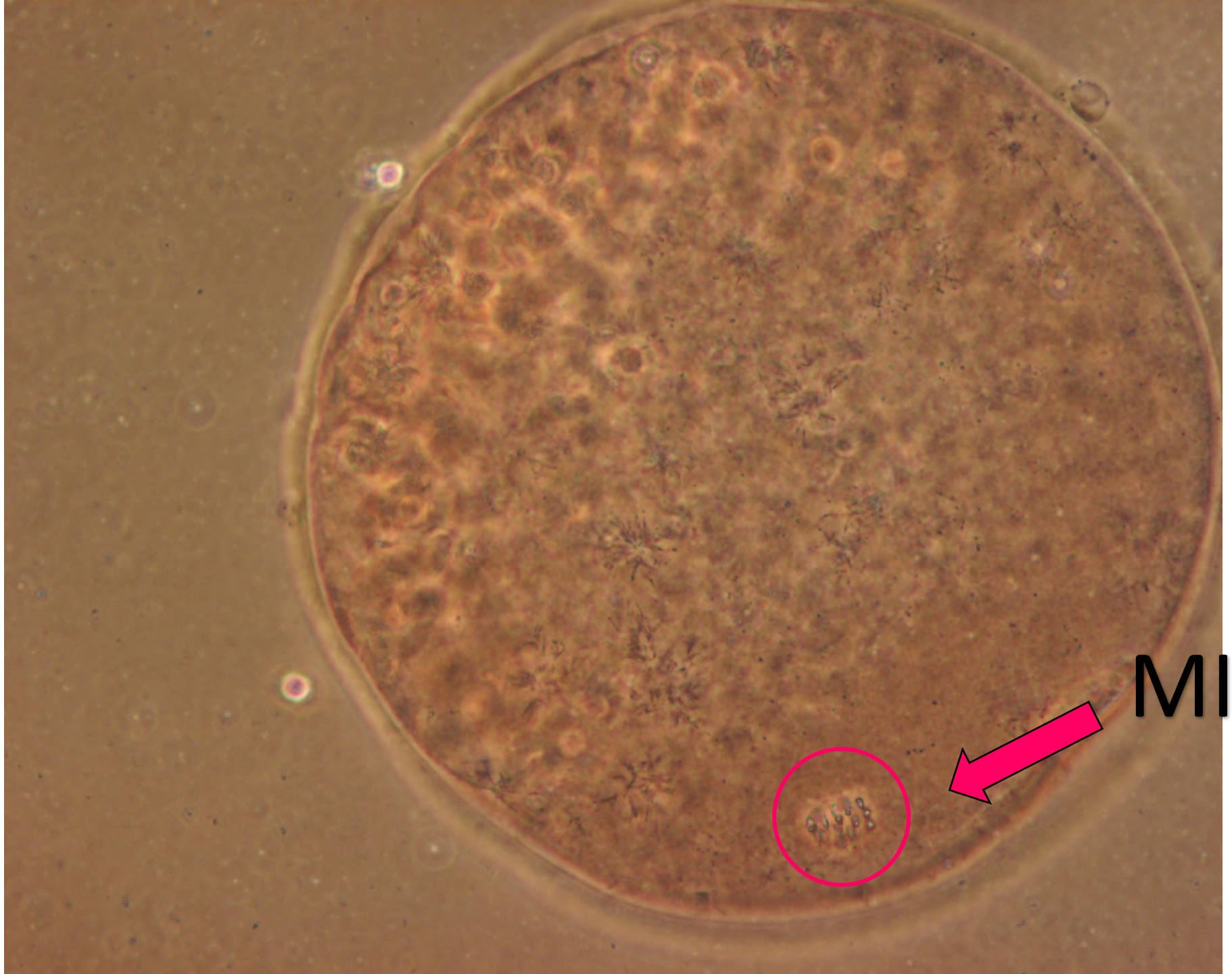
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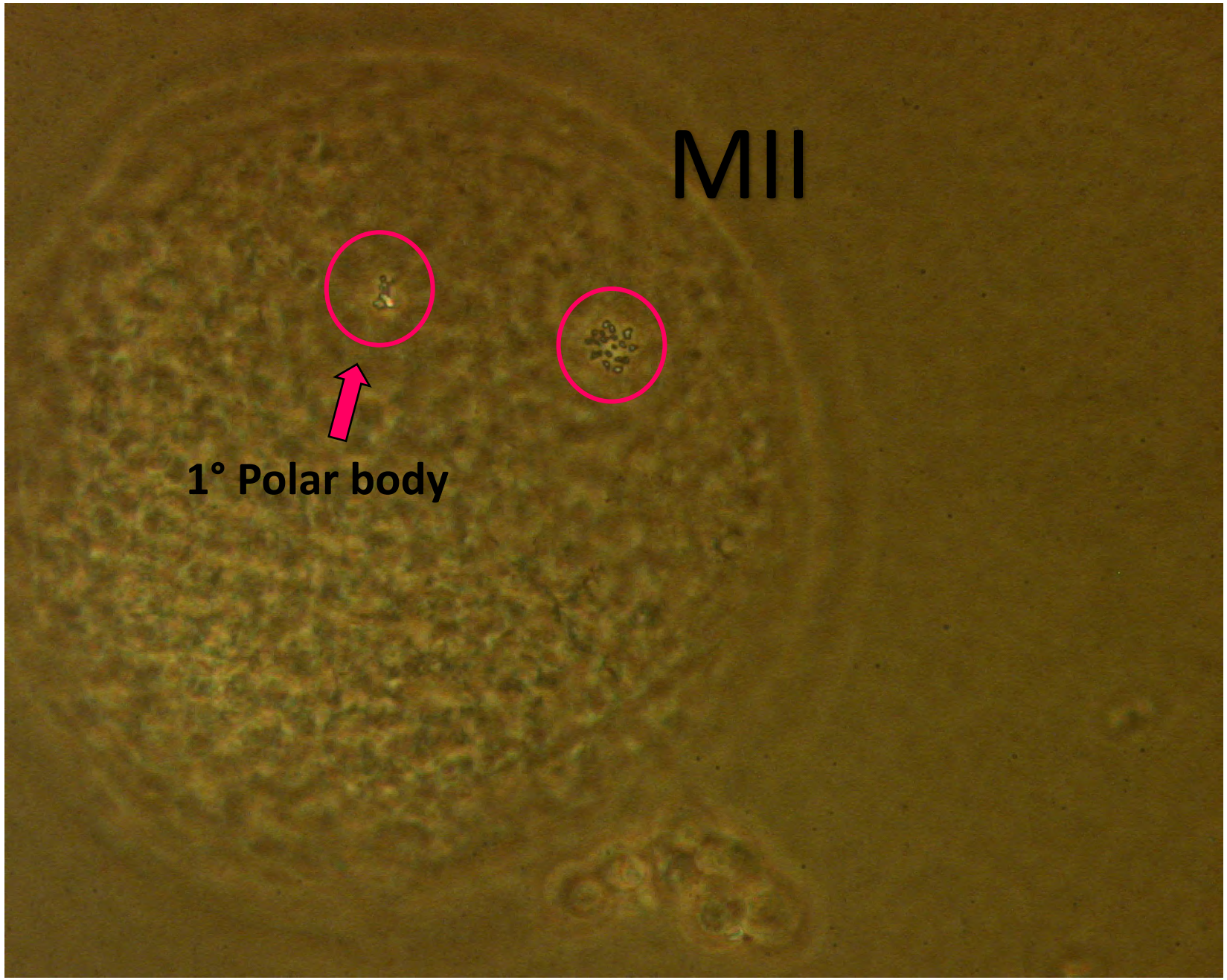
MII
1° Polar body


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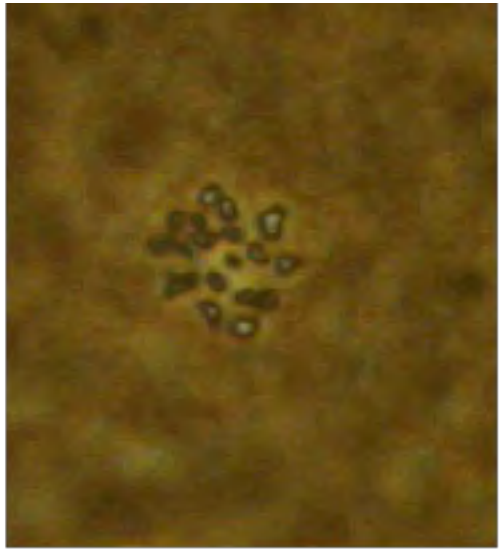

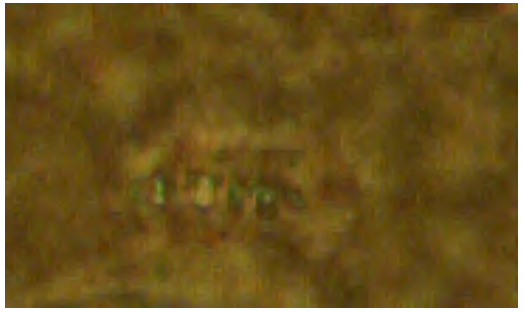

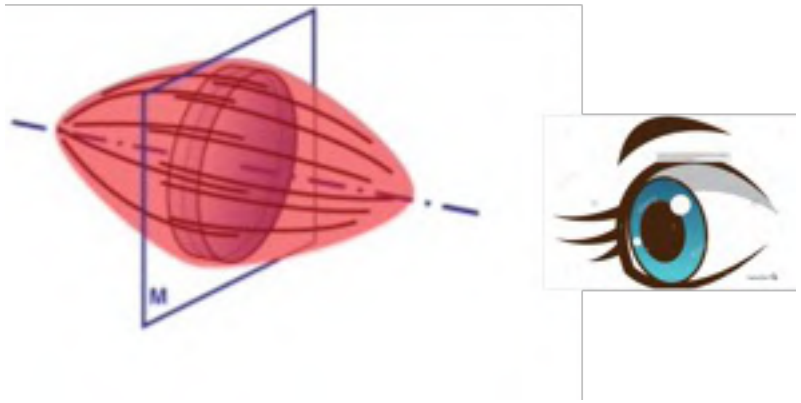
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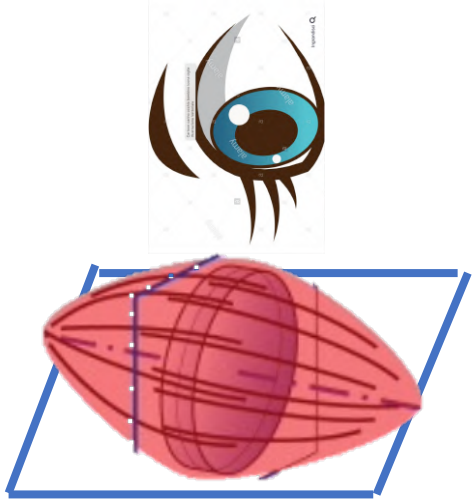

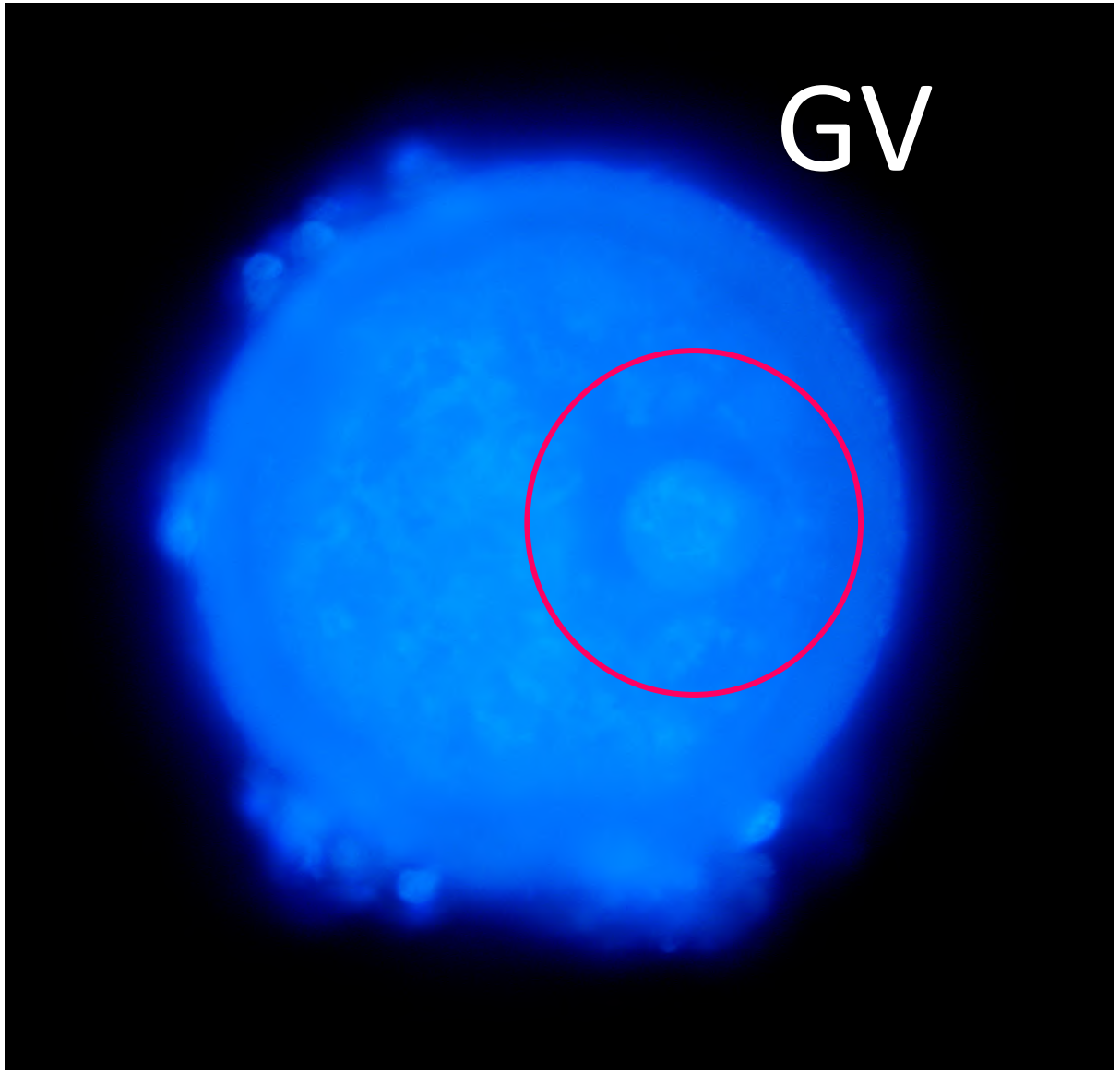
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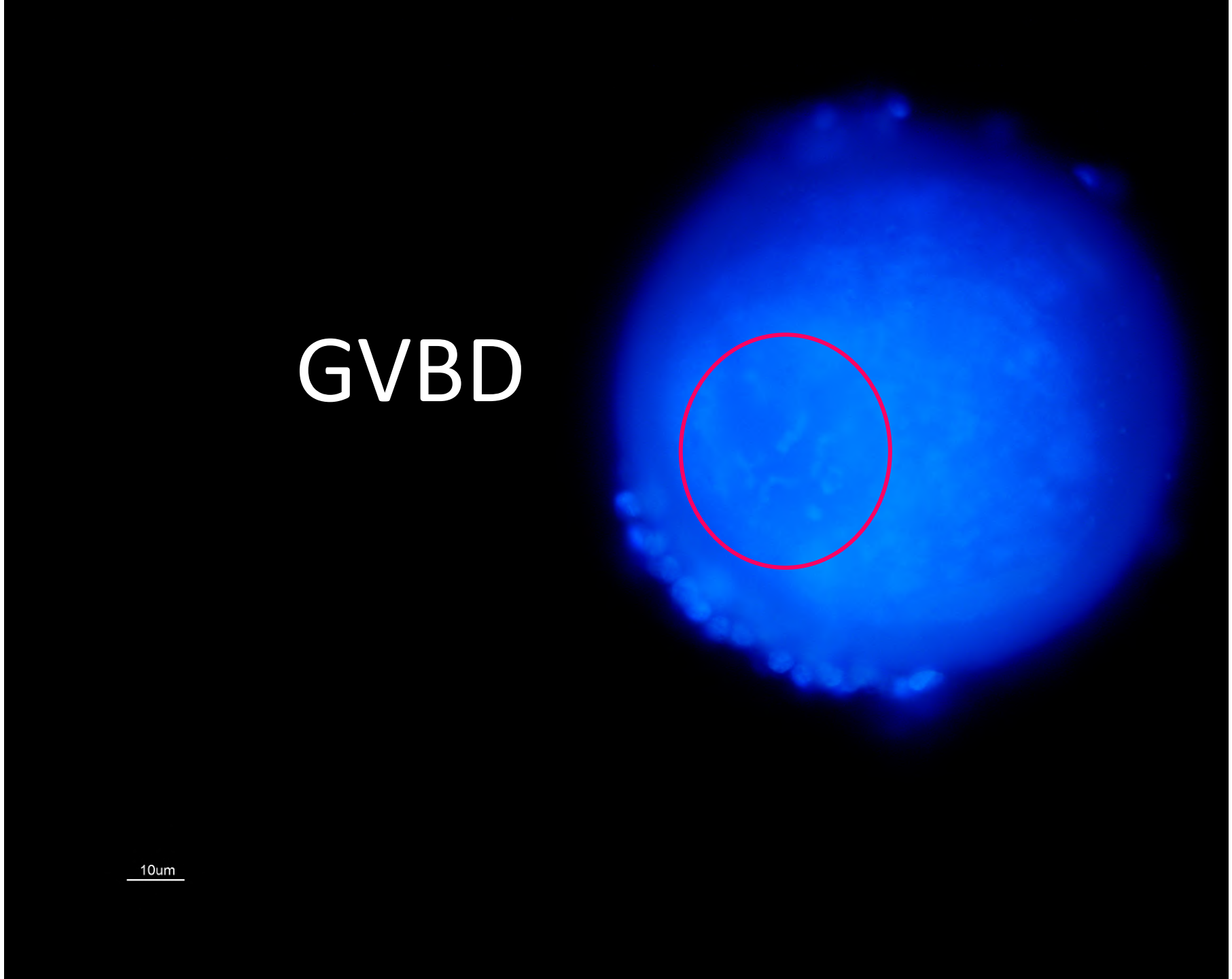
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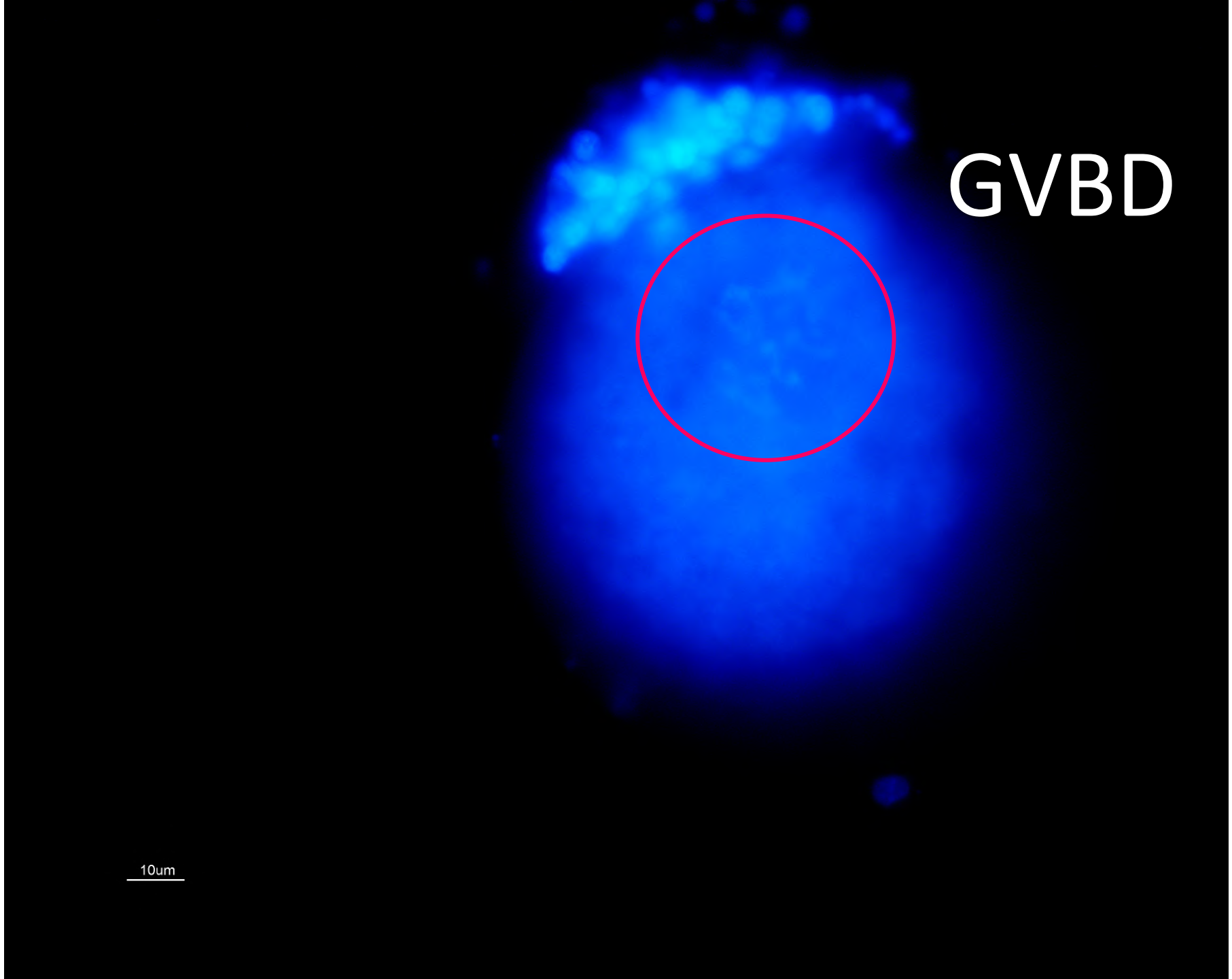
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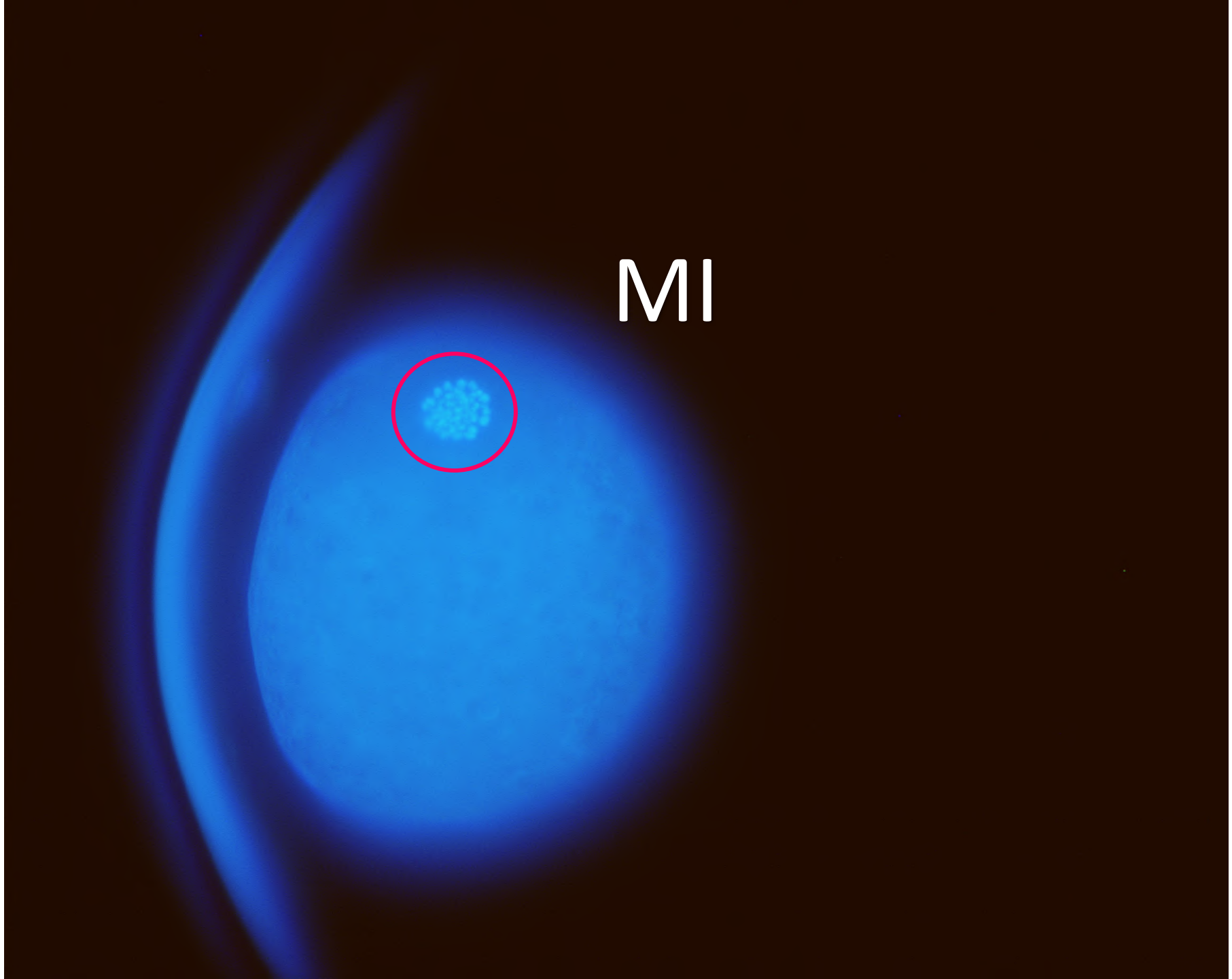
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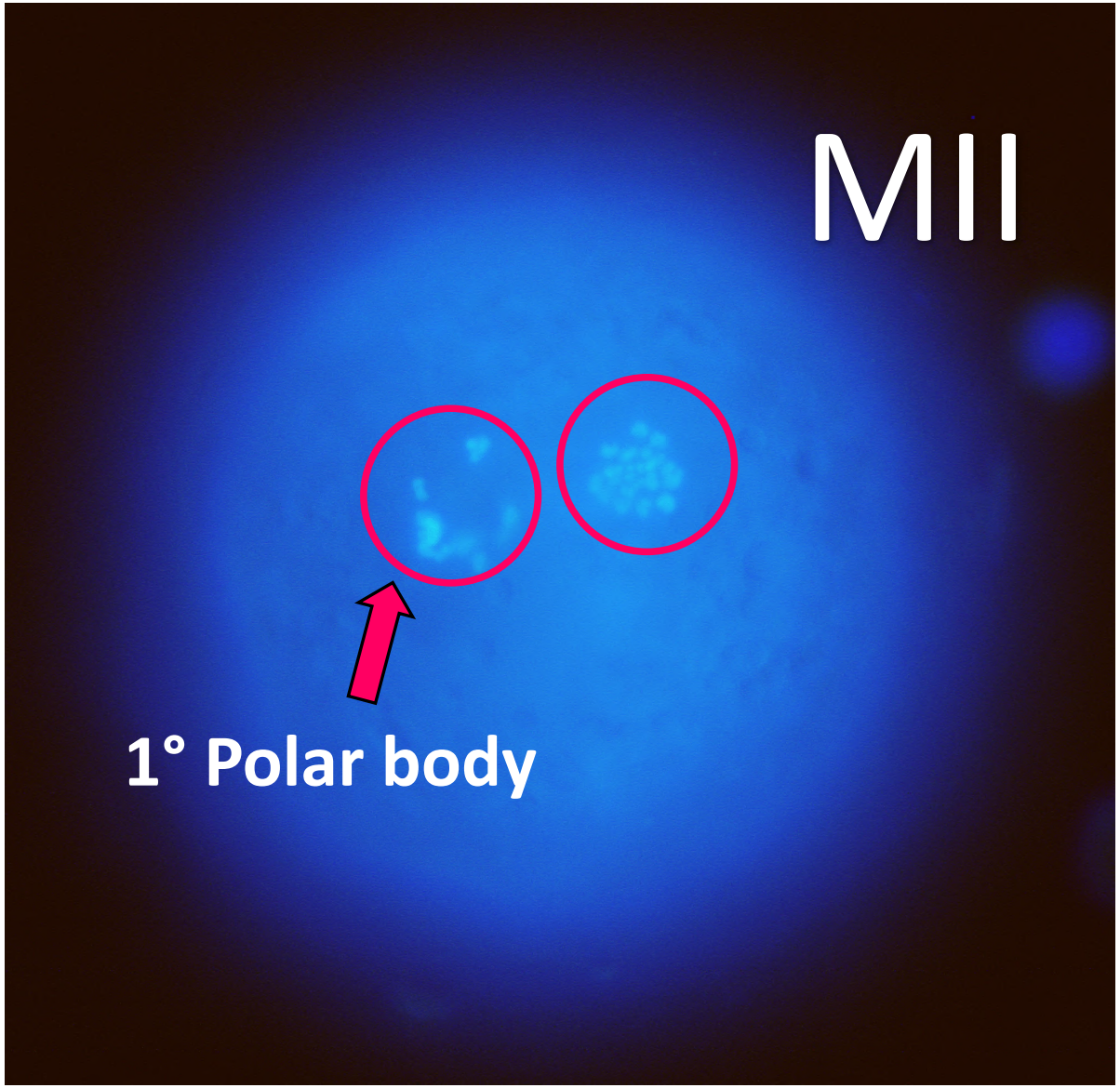
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