Annex I - Images

Set 1:

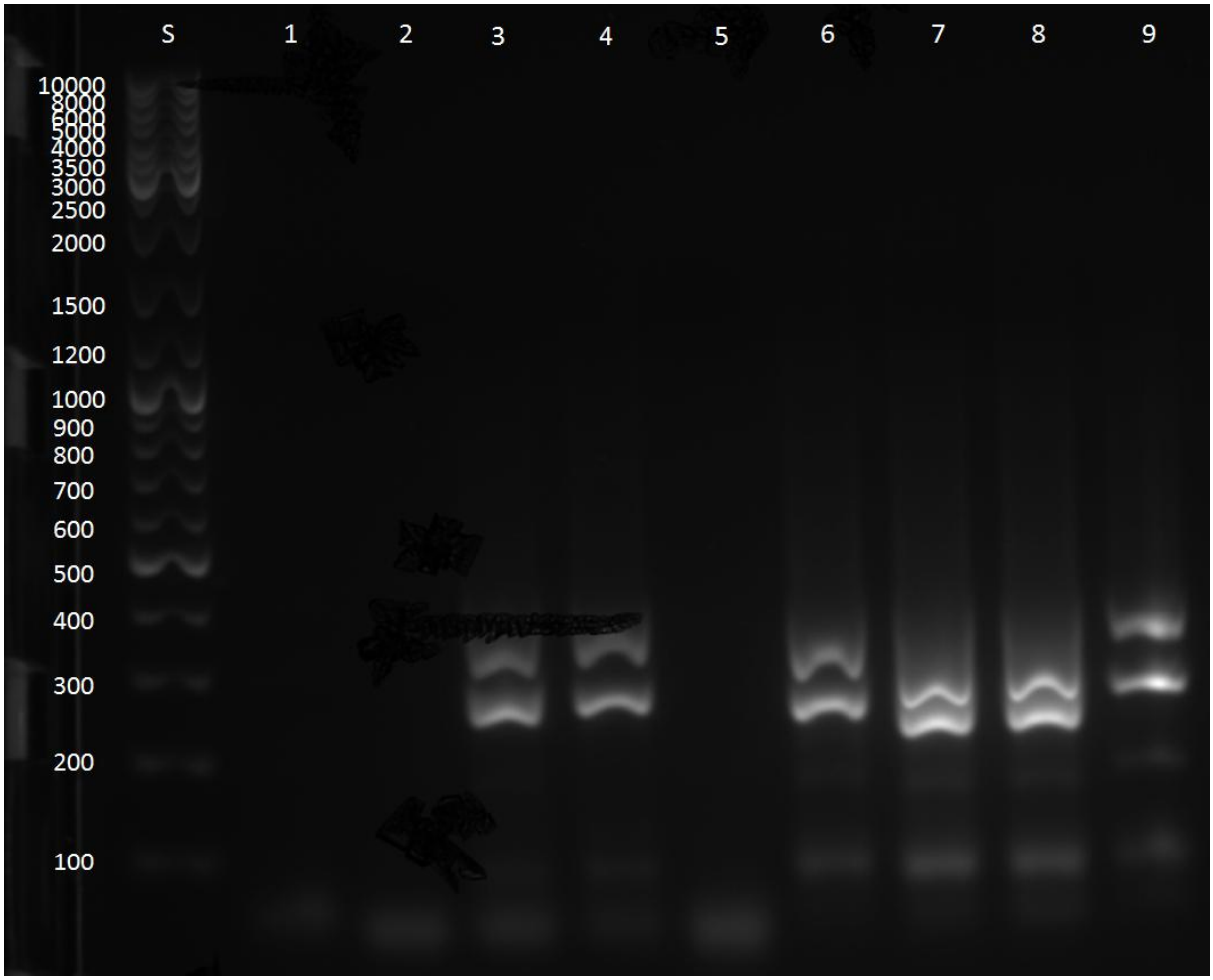

Image 1: Quality Control PCR of Whole Genome Amplification. Agarose gel electrophoresis image showing the product of Quality Control PCR of whole genome amplification products. Lane S shows a DNA staircase ranging from 10000 bp to 100 bp. Lanes 1 to 9 correspond with the content of PCR tubes from whole genome amplification, after undergoing GoTaq® Control PCR. Lane 1 contains negative control (solution without cells), lanes 2, 3, 4 and 5 contain single cells, lane 6 contains a pool of 15 cells, lane 7 contains a pool of 10 cells and lane 8 contains a pool of 10 cells. Lane 9 contains the positive control (Human Genomic DNA: Female (Promega)). The black lines had been painted with a pen only visible under ultraviolet light and could not be removed through digital editing without affecting the interpretation of the gel.

Set 2: Images from fluorescent microscopy. 09/08/2016 and 29/08/2016. Colours are digitally assigned depending of the channel the fluorescence was detected on: white to DAPI channel, yellow to Cy3 and red to Cy5. DAPI stains DNA, thus marking nuclei. Cy3 was conjugated to detection probes which should recognize β-actin transcript presence via circularised padlock probes. Cy5 was conjugated to detection probes which should do the same for Androgen Receptor Full Length (AR-201) transcript.

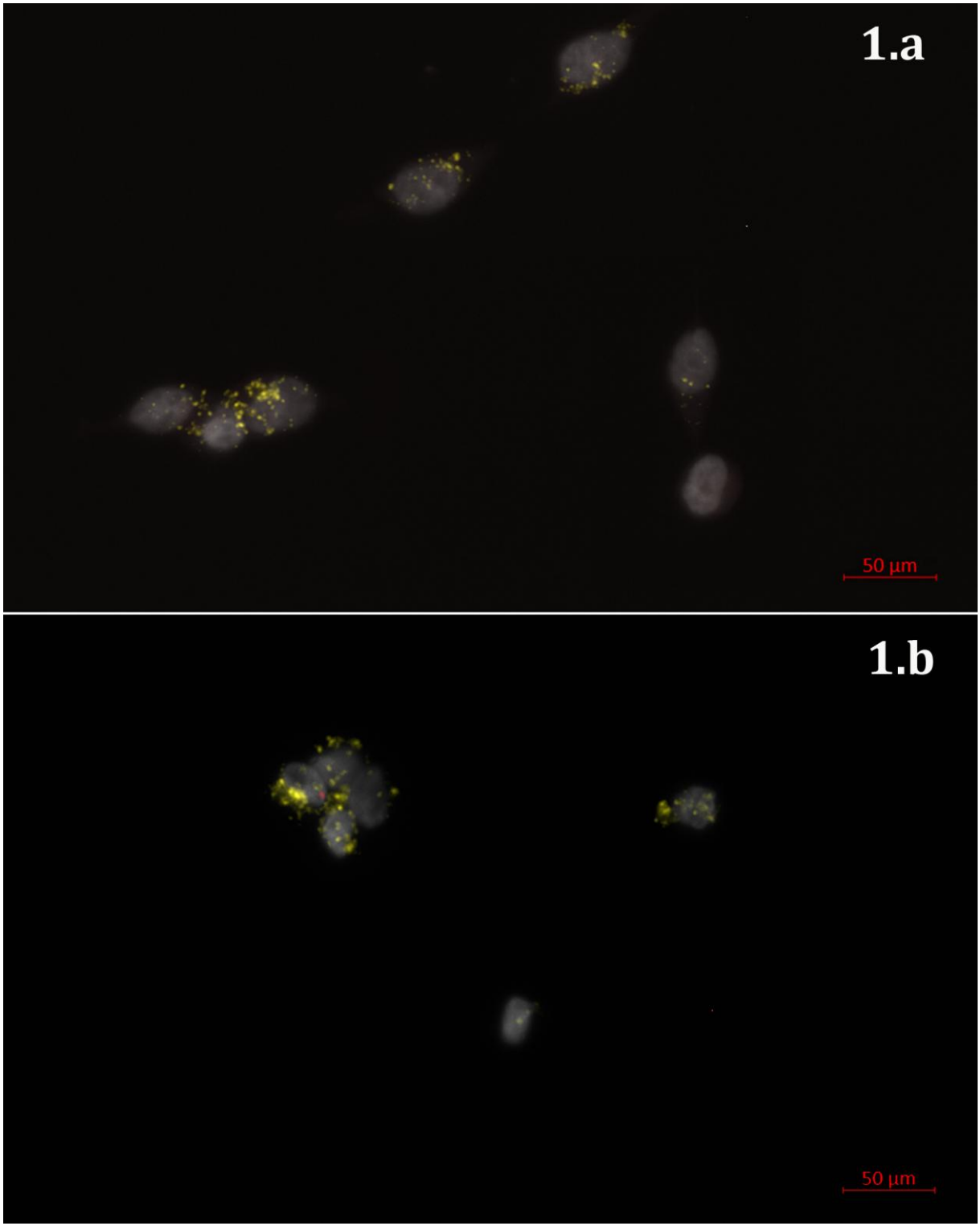

Image 1: LNCaP from in situ padlock probing assay 9th of August 2016. 1.a: LNCaP seeded on slide. 1.b: LNCaP in suspension.

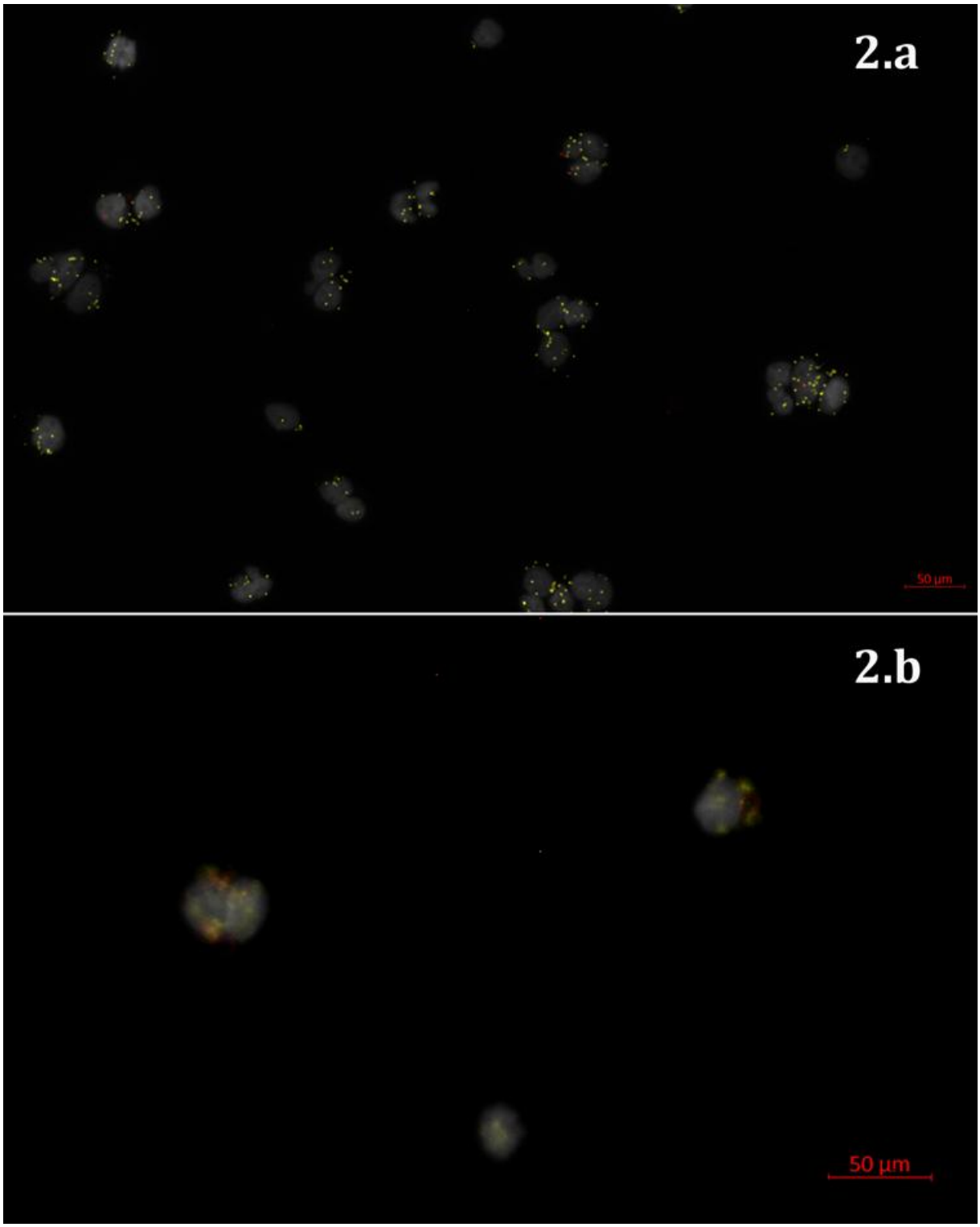

Image 2: VCaP from *in situ* padlock probing assay 9th of August 2016. 2.a: VCaP seeded on slide. 2.b: VCaP in suspension.

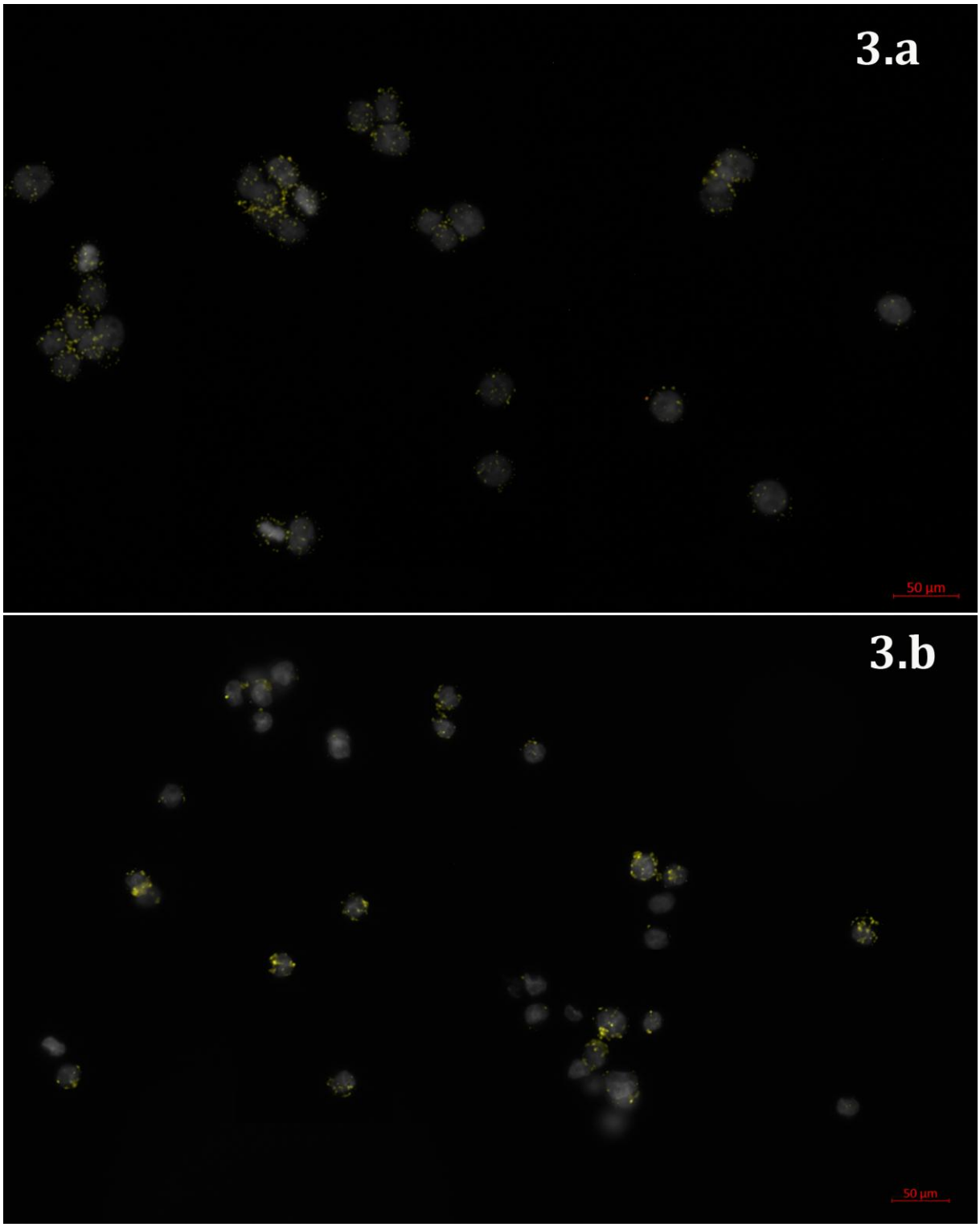

Image 3: HT-29 from *in situ* padlock probing assay 9th of August 2016. 2.a: HT-29 seeded on slide. 2.b: HT-29 in suspension.

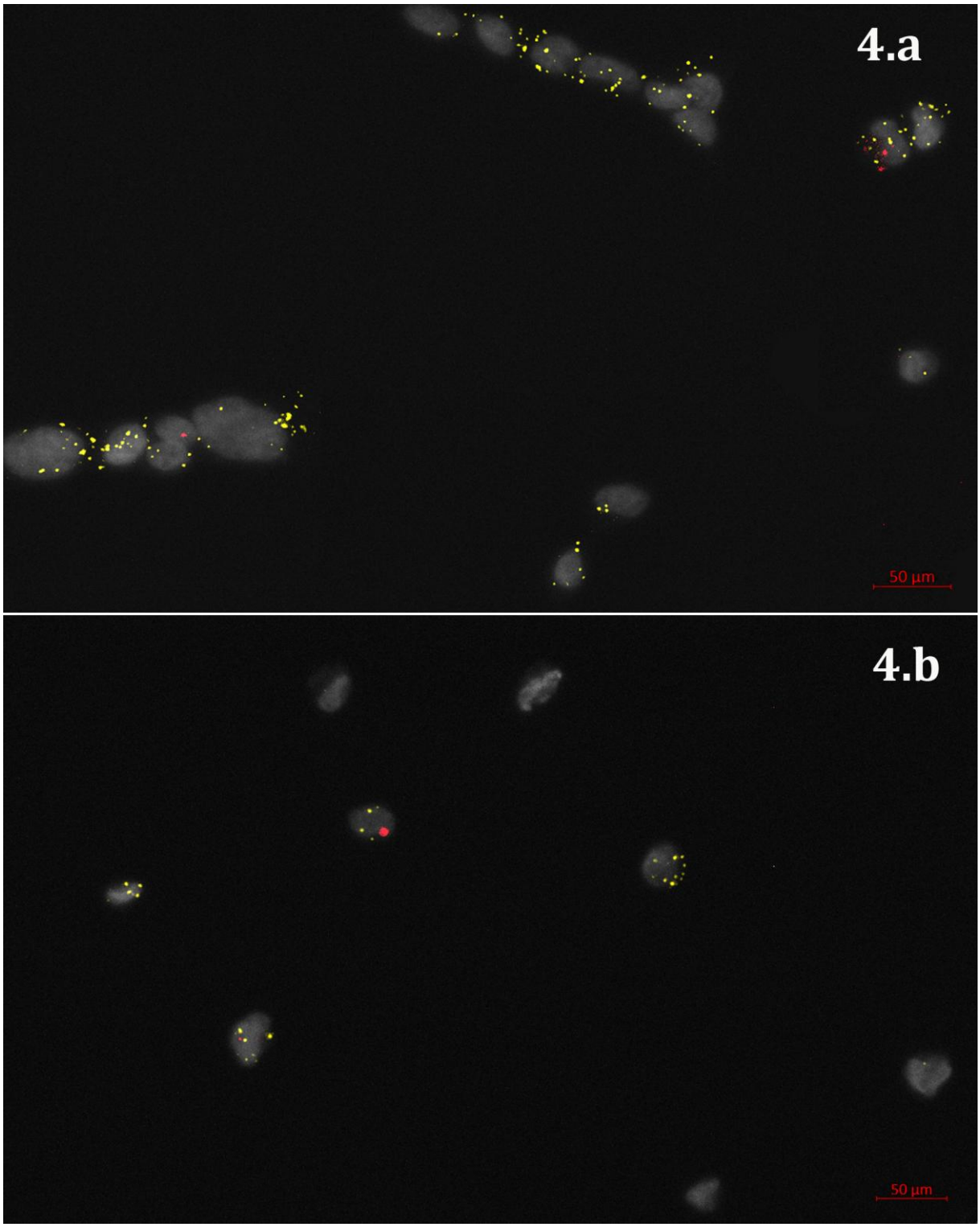

Image 4: LNCaP from *in situ* padlock probing assay 29th of August 2016. 4.a: LNCaP seeded on slide. 4.b: LNCaP in suspension.

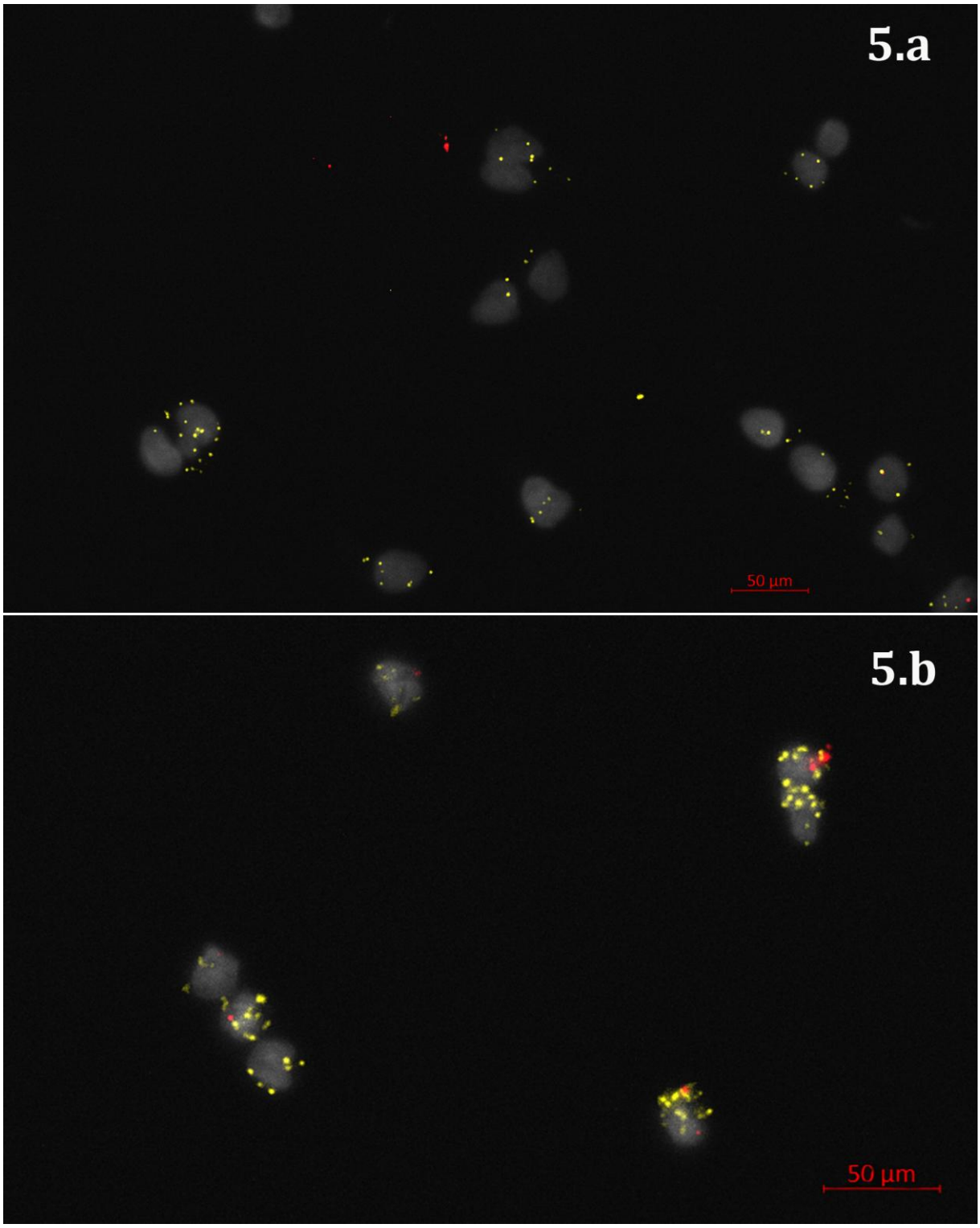

Image 5: VCaP from *in situ* padlock probing assay 29th of August 2016. 5.a: VCaP seeded on slide. 5.b: VCaP in suspension.

Set 3: Explanatory images for Cell Profiler Quantification and Exclusion rules, bigger size. HT-29 cell line which underwent *in situ* padlock probing seeded on a slide. The image was taken on 10th of August 2016.

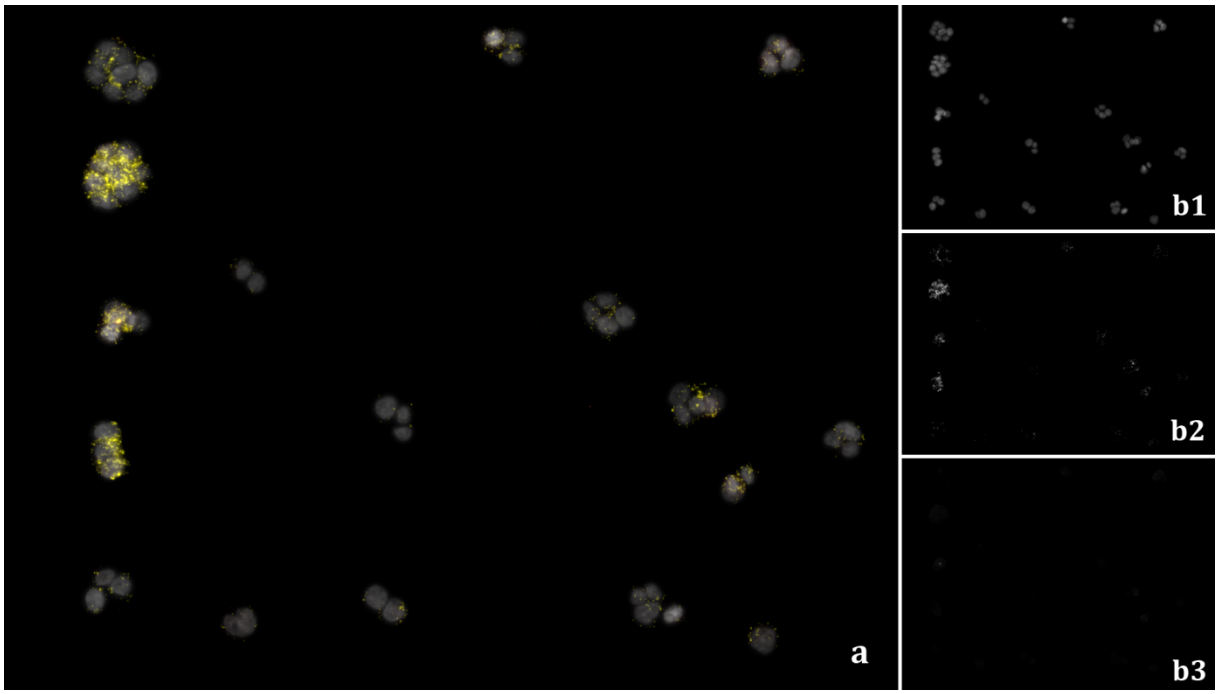

Image a: Fluorescence microscopy image. It contains three different layers, one for each fluorophore. Nuclei (DAPI) in grey, β-actin expression (Cy3) in yellow and androgen receptor full length expression (Cy5) in red. Image b1: Fluorescence microscopy image. It contains only fluorescence detected through DAPI channel. Image b2: Fluorescence microscopy image. It contains only fluorescence detected through Cy3 channel. Image b3: Fluorescence microscopy image. It contains only fluorescence detected through Cy5 channel.

Image c: Output image showing the quantification results on it. Cytoplasm areas are delimited by aquamarine perimeters and surround what CellProfiler identified as a single nucleus. Yellow areas encircle what cell profiler identified as a single Cy3 fluorescent dot and red areas encircle what CellProfiler identified as a single Cy5 fluorescent dot. The number of dots counted in each cytoplasm is noted in yellow (Cy3) and red (Cy5) figures. Image d: Image c with explanatory markers added. Thick circles mark the cases where the first rule for exclusion was applied: Red circles surround cases where the measures were excluded from quantification, while green circles surround cases where the measures could not be excluded but were manually revised. Purple lines differentiate manually traced lines from those traced by CellProfiler.

Annex II – Additional information

List of initials used throughout the thesis:

- DEPC: Diethyl pyrocarbonate. RNases are ubiquitous and must be inactivated to protect the mRNA transcripts that the assay is targeting. Diethyl pyrocarbonate inactives RNases.
- DEPC-PBS: Diethyl pyrocarbonate combined with phosphate-buffered saline, which maintains an isotonic osmolarity.
- DEPC-H_2O: Diethyl pyrocarbonate diluted in water.
- DEPC-PBS-Tween: DEPC-PBS with 0.05% Tween-20 (Sigma). Tween-20 is a detergent that solubilises hydrophobic substances.
- 0.1 M HCl-DEPC-H_2O: HCl (Sigma) diluted in diethyl pyrocarbonate and water to 0.1 mol of HCl per solution litre.
- SNP: Single Nucleotide Polymorphism
- PCR: Polymerase Chain Reaction
- WGA: Whole Genome Amplification
- DAPI: 4',6-diamidino-2-phenylindole

Cell culture medium components:

VCaP media consisted of DMEM high glucose (Gibco, Invitrogen, Paisley, UK) supplemented with 10% FCS (Fetal Calf Serum) and 1% P/S (Penicillin/Streptomycin). LNCaP media consisted of RPMI1640 (Gibco, Invitrogen, Paisley, UK) supplemented with 10% FCS (Fetal Calf Serum) and 1% P/S (Penicillin/Streptomycin). HT-29 media consisted of McCoy's Medium supplemented with FBS (Fetal Bovine Serum), HEPES (hydroxyethyl piperazineethanesulfonic acid), L-Glutamine and P/S (Penicillin/Streptomycin).

In situ reverse transcription master mix composition:

Reverse transcription master mix composition: 20 U/ µl TranscriptMe Retrotranscriptase (DNA-Gdansk, Gdansk, Poland), RT buffer (Gdansk), 1U/µl RiboLock RNase Inhibitor (Thermo Fisher Scientific, Waltham, MA, USA), 1 µM locked nucleic acid primer targeting AR-201 (Exiqon, Vedbaek, Denmark), 1 µM locked nucleic acid primer targeting β-actin (Exiqon), 0.5 mM dNTPs (Thermofisher Scientific), 0.4 µg/µl BSA and DEPC-H_2O.

In situ ligation master mix composition:

Ligation master mix: 0.5U/µl Ampligase (Epicentre, Illumina, Madison, WI, USA), Ampligase buffer (Epicentre, Illumina), 0.4 U/µl RNase H (Thermofisher Scientific), 0.4 µg/µl, BSA, 0,05 M KCl, 20% formamide (Sigma), 0.1 µM padlock probe targeting AR-201 cDNA (Integrated DNA Technologies, Coralville, IA, USA), 0,1 µM padlock probe targeting β-actin cDNA (Integrated DNA Technologies) and DEPC-H_2O.

In situ rolling circle amplification master mix composition:

Rolling Circle Amplification Master Mix: 1U/µl Φ29 polymerase (Thermofisher Scientific), Φ29 polymerase buffer (Thermofisher Scientific), 0.25 mM dNTP (Thermofisher Scientific), 0.4 µg/µl BSA, 5% glycerol (Sigma) and DEPC-H_2O.

Detection probe hybridization master mix composition:

Detection Master Mix: Hybridisation buffer, 0.1 µM detection oligonucleotide complementary to Lin16 conjugated with fluorophore Cy3 (Biomers, Ulm, Germany), 0.1 µM detection oligonucleotide complementary to Lin33 conjugated with fluorophore Cy5 (Biomers), 2 µg/ml DAPI (Thermofisher Scientific) and DEPC-H_2O.

GoTaq® Green master mix composition:

GoTaq® Green Master Mix is composed of: GoTaq® DNA Polymerase, Green GoTaq® Reaction Buffer, dNTPs and $MgCl_2$.