

# Sensors for Fertility Monitoring of Cows

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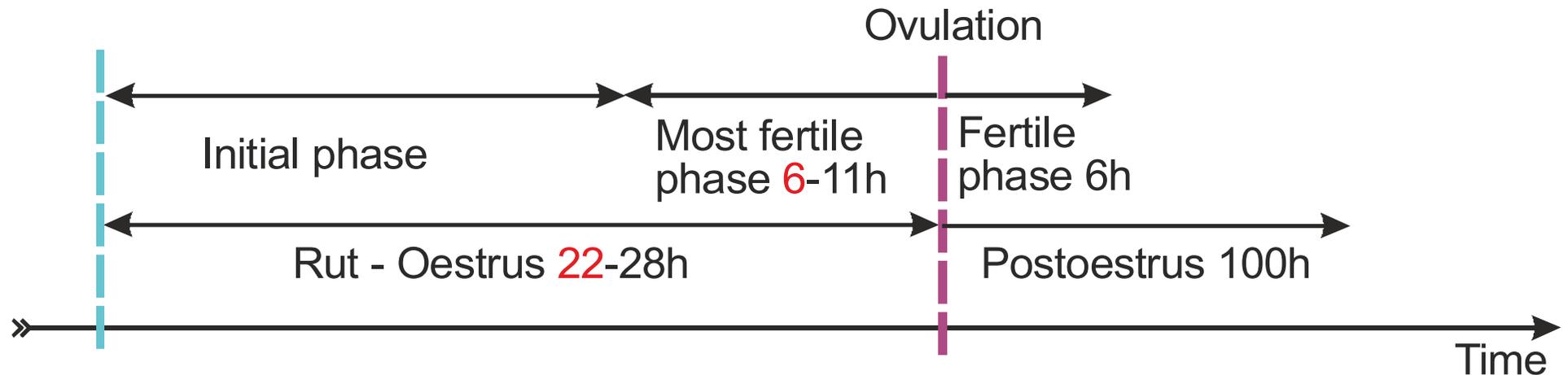


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# Introduction

1. The functional state of fertility is determined by:
  - optimum fertility,
  - health of the vaginal duct.
2. Nowadays, the oestrous cycles of cows are often resynchronized. Cows with high milk production have:
  - shorter duration of oestrus,
  - lower intensity signs of oestrus periods,than lower producing cows measured at the same conditions.
3. An important problem of cow reproduction is keeping the health of the vaginal duct.
  - when the vaginal changes are visible the illness is usually seriously advanced,
  - the bacterial vaginitis can be in its initial and hidden state invisible.
4. Usual medical techniques, like
  - the hormone test in urine or milk and
  - the liquid based cytology,are not automated are not ideal when farm environment is considered.
5. Sensing of cow functional state of fertility is important for the cattle and dairy industries. There is a need to develop improved and new methods.
6. The method presented in this paper is based on fiber optic capillaries techniques using neural network analysis. We have investigated the vaginal fluids of healthy cows and of those suffering from vaginitis and have proved that the method allows establishing the oestrous state and distinguishing between healthy and sick animals.

# Cows oestrus



Cows with high milk production ( $\geq 40$  kg/day) have:

- shorter duration of oestrus ( $6.2 \pm 0.5$ h versus  $10.9 \pm 0.7$ h),
- less total mounts per cow ( $6.3 \pm 0.4$  versus  $8.8 \pm 0.6$ )
- shorter duration of total time standing to be mounted ( $21.7 \pm 1.9$ h versus  $28.2 \pm 1.9$ h)

than lower producing cows measured at the same conditions.

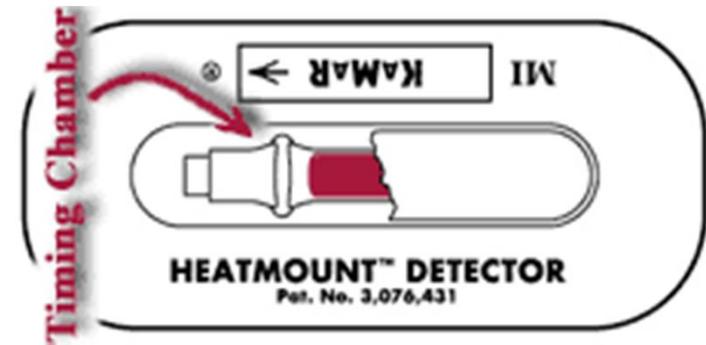
# Cows oestrus determination techniques

1. Visual observation
  - Cow stands to be mounted
  - Cow mounts other cows
  - Increased walking
  - Restlessness
  - Chin pressing
  - Swelling of vulva & mucus discharge
2. Wearable collar systems
3. Health (temperature) monitoring pill
4. Hands-on veterinary
  - In-situ examinations (Vaginal duct examination)
  - In-lab examinations (Sensor of milk or urine progesterone, cytology)

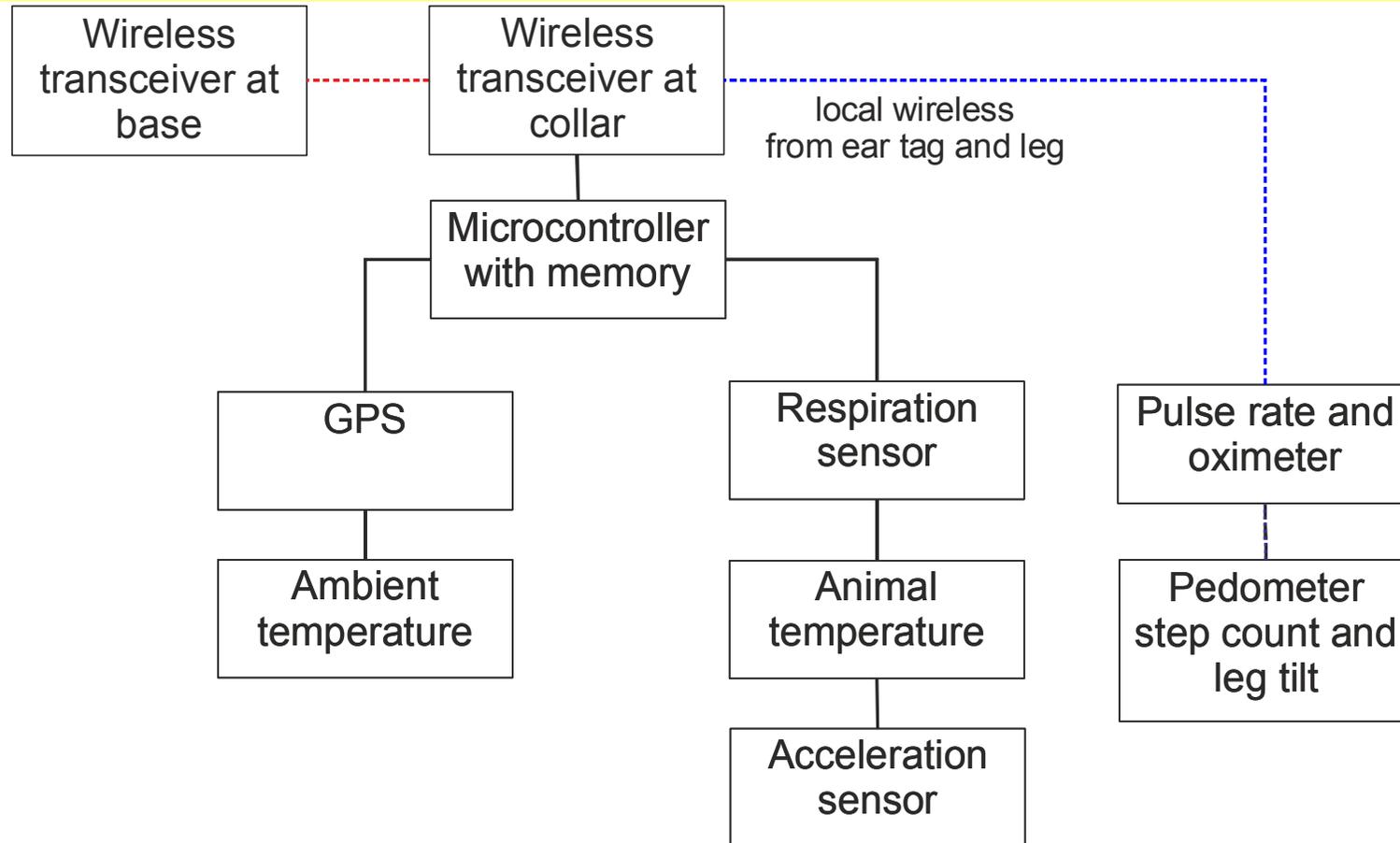
# The simplest devices - tail painting detectors

Adhesive tail head patches of detectors are glued onto the top of the cows tail (sacrum, tail head).

1. Kamar detectors are pressure sensitive devices activated by pressure from the brisket of a mounting animal. When the cow is mounted the applied pressure changes the colour of the chemical detector from white to red.
2. EstroTect Patches detectors use rubbing surfaces similar to „lottery ticket”. When the cow is mounted the surface is peeled off, leaving the bright, easily detectable fluorescent color behind.



# Automatic devices - construction of collar sensors systems for cattle monitoring



- The most important issue of collars is reduction of device power consumption while the data have to be continuously collected when cattle is on the pasture.
- The reduction of power consumption is realized by adaptive sensing and wireless transmission of data between collars on cattle necks and base station when a cattle are in the paddock.

# Automatic devices - collar sensor systems

- Fertility determination of cows
  - Temperature-sensor: temperature cycles
  - Pedometer: number of step is specific oestrus behavior
  - Acceleration sensor: restlessness of the cow
- State of health determination of cattle:
  - Cattle basic diagnostic data, such as temperature of body, respiration rate, blood oxygen saturation and pulse rate are continuously monitored and locally stored. If rapid changes happen, the alarm signal is transmitted to the base.
- Virtual fencing technology - monitoring of cattle location and movements:
  - If a cow approaches a virtual fence the collar emits a warning sound, and if it continues past the fence line the cow receives a tactile stimulus. In trials cattle quickly learn to react to the audio warning and stop before the virtual fence.
- Activity of cattle monitoring
  - Cattle behavior control as ruminating, sleeping and grazing is possible using an accelerometer, a pedometer and a GPS.



T. Wark, et al. "The Design and Evaluation of a Mobile Sensor/Actuator Network for Autonomous Animal Control". Proc. of IPSN, Cambridge (MIT Campus), USA, April 2007.  
L. Nagl, et al.. „Wearable Sensor System for Wireless State-of-Health Determination in Cattle”, Proc. 25th Ann Int Conf of the IEEE Engin in Med and Biol Soc. 25:3012-3015, 2003

# Disadvantages of present collar sensors systems for oestrus detection

1. Temperature sensors have not worked very well because of animal body temperature variations.
  - Cows allow their temperature to rise and fall with environmental conditions,
  - The temperature in the stomach can quickly change when a cow drinks.
2. Pedometer data of cattle have to be corrected with:
  - Movement of animals from barn to different fields.
3. Acceleration data of cattle have to be corrected with:
  - presence of bugs as mosquitos, flies and horse-flies,
  - high ambient temperatures causing heat stress result in physical lethargy and reduced oestrus detection efficiency in cows,
  - housing, floor surface, yield, lameness and number of herd mates in oestrus simultaneously



There is a potential of data mining of individual cow and multiparametric data to determine reproductive heat cycle time. For this purpose the data of pedometer, temperature and position (GPS) can be analyzed together.

# Sensors for detecting functional state of cows

1. Sensors positioned in the nose.
  - Breath sensor using 6 of Si integrated gas sensors of food/smoke, air quality, ammonia, hydrocarbons, alcohols, two independent sensors of temperature and humidity. The information processed with artificial neural network (ANN). The health of the cow was judged against blood samples. The identification rate of samples was 76%.
2. Sensor positioned in stomach.
  - Heart rate (acoustic sensor) and temperature sensors are used. The abnormal temperature and heart rate may be correlated with mad cow disease.

**The functional state of cow selected for insemination has to be monitored in different way.**

1. J.W.Gardner, E.L.Hines, F.Molinier, N. Bartlett and T.T.Mottram, „Prediction of health of dairy cattle from breath samples using neural network with parametric model of dynamic response of array of semiconducting gas sensors”, IEE Proc'.-Sci. Meas. Technol., Vol 144(2), 1999, DOI 10.1049lipsml:1 9990100.
2. A. Martinez. "Acquisition of Heart Rate and Core Body Temperature in Cattle Using Ingestible Sensors," Electrical & Computer Engineering. Manhattan, KS: Kansas State University, 2007.
3. T. Wark, P. Corke, P. Sikka, L. Klingbeil, Y. Guo, C. Crossman, P. Valencia, D. Swain, and G. Bishop-Hurley, „Transforming Agriculture through Pervasive Wireless Sensor Networks”, Pervasive computing IEEE Computer Society, vol. April-June, 2007, pp. 50-57

# The simplest veterinary examination - vaginal fluid visual examination

1. The fluid is typically clear. Its clarity depends on the phase of the fertility cycle, the presence of an infection, certain drugs, genetic factors and diet.
2. The vaginal fluid can be present:
  1. at ovulation, few days before ovulation - vaginal fluid is transparent and ductile,
  2. sometimes at full cycle - vaginal fluid is turbid, milk white, volume is very low <50ml,
  3. at full cycle when cow has vaginitis - vaginal fluid is transparent,
  4. at full cycle when cow has uterus suppurative matter - vaginal fluid is turbid, yellowish or greenish and stinking,
  5. at certain sexually transmitted diseases - vaginal fluid can vary in consistency, texture and color.

**Therefore, vaginal fluid visual examinations are not sufficient for fertility phase or vaginitis diagnose.**

# The simplest veterinary test - vaginal fluid pH

1. Cow vaginal fluid include pyridine, squalene, urea, acetic acid, lactic acid, aliphatic acids, complex alcohols, glycols (including propylene glycol) ketones, and aldehydes.
2. The vagina's aliphatic acids come from the metabolic processes of resident bacteria, including Lactobacilli.
3. The normal pH of vaginal fluid is between 3.8 and 4.5. (vaginal fluid is slightly acidic). The vaginal fluid can become more acidic:
  1. when cow has vaginitis
  2. when cow has certain sexually transmitted diseases
  3. when the cow is in rut - for all mammals the acid content varies with the menstrual cycle, rising from day one after menstruation and peaking mid-cycle, just before ovulation.

**Therefore, the pH are not sufficient for fertility phase or vaginitis diagnose.**

# Our objectives

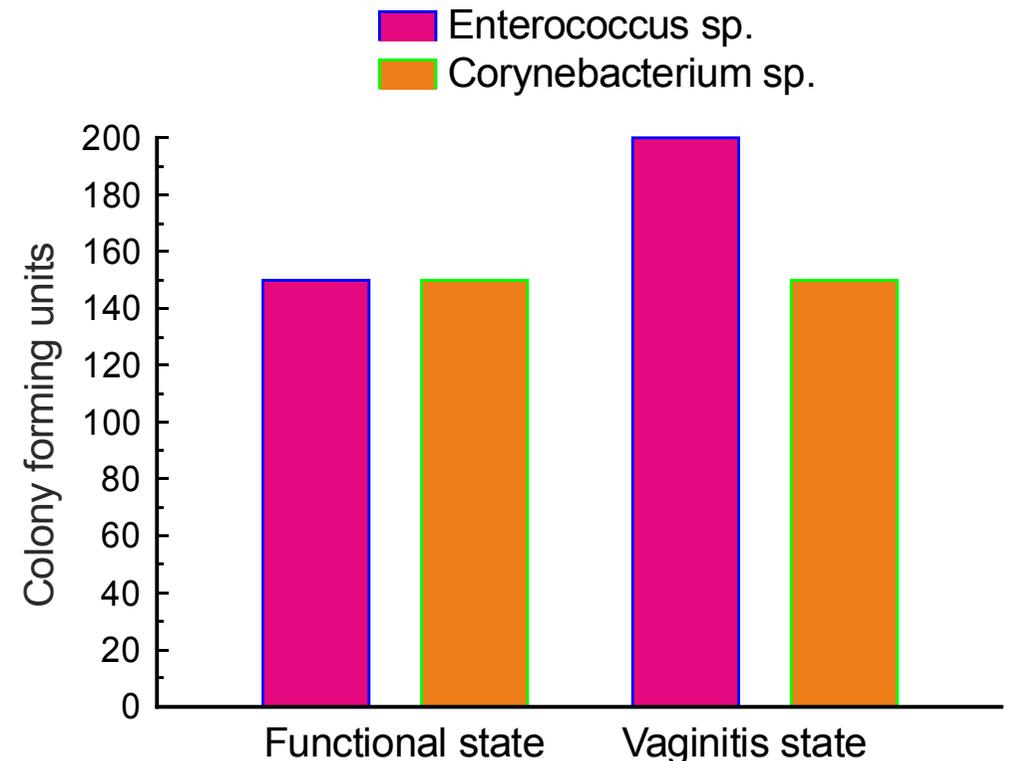
1. To investigate and develop a sensor which would provide the information of functional state of fertility useable to make decisions on insemination of the cows with a higher sensitivity and specificity than the existing construction.
2. To develop a sensor which also could be operated in the farm environment, not only in laboratory environment.
3. Development of a method that would yield itself easily to automation, miniaturization and be of low cost.

# Our assumptions

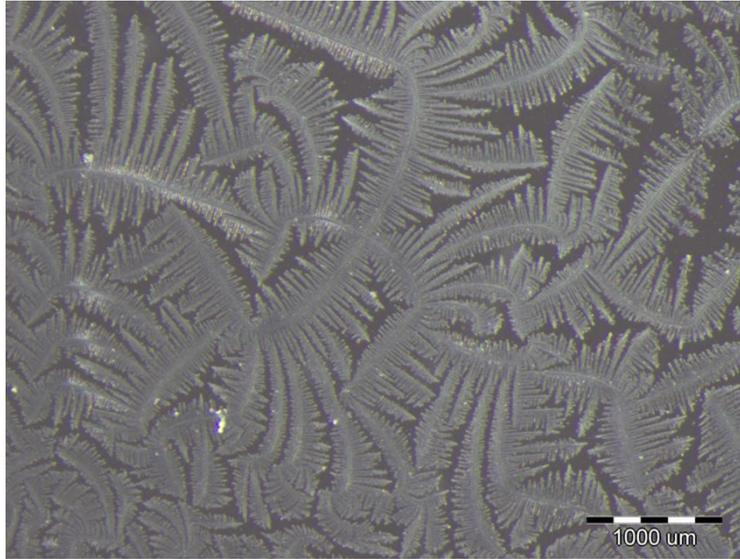
1. The vaginal fluid parameters which are connected with fertility and health of vaginal duct could be examined with optoelectronic methods.
2. The multi-parametric sensing is required because of vaginal fluid structure. The sensing of functional fertility can require additional influence on collected sample:
  - mechanical,
  - optical,
  - thermal.
3. The volume of vaginal fluid sample is limited, we examine  $10\text{mm}^3 = 10\mu\text{l}$ .
  - Optical capillaries will be used as disposable vaginal fluid container and sensor optrodes.

# Collection of the vaginal fluid of cows

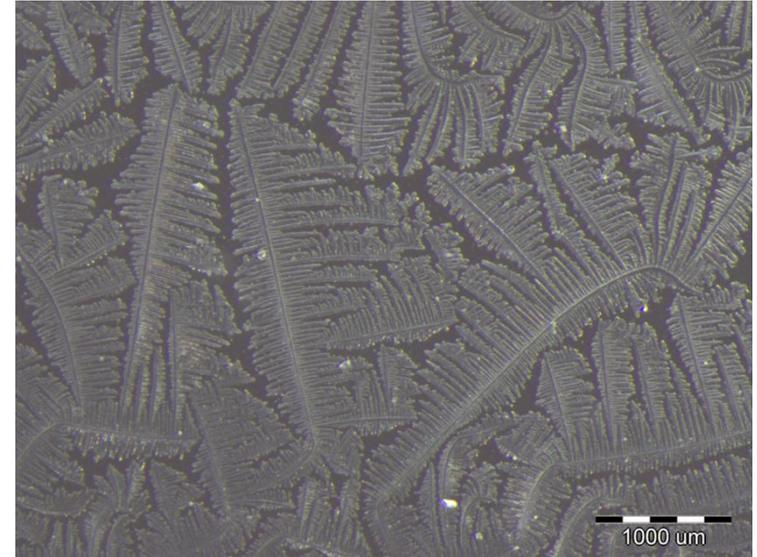
1. We analyzed vaginal fluids of Holstein-Friesian and Jersey cow breeds that are typical cattle in Poland.
2. The cows were selected randomly from herds counting from 10 to 100 animals.
3. We examined samples of 15 cows classified as healthy. The vaginal fluid was collected in oestrus and the post-oestrus state.
4. The vaginal fluid was also collected from 2 cows which were in oestrus and had vaginitis diagnosed by a veterinary doctor on the farm and confirmed by microbiological analysis.
5. The vaginal fluid was also collected from cow which was in oestrus and had no ovulation.



# Drying of the vaginal fluid - arborization test



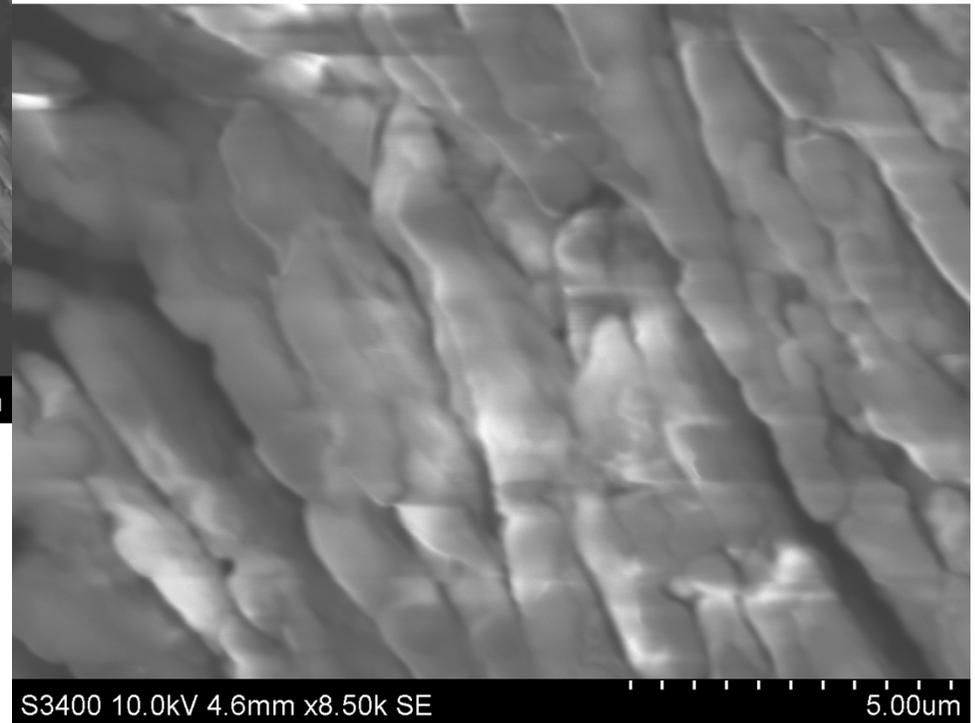
Most fertile period with ovulation  
(normal state)  
- cow is fertile



Most fertile period but without  
ovulation (pathological state)  
– cow is infertile

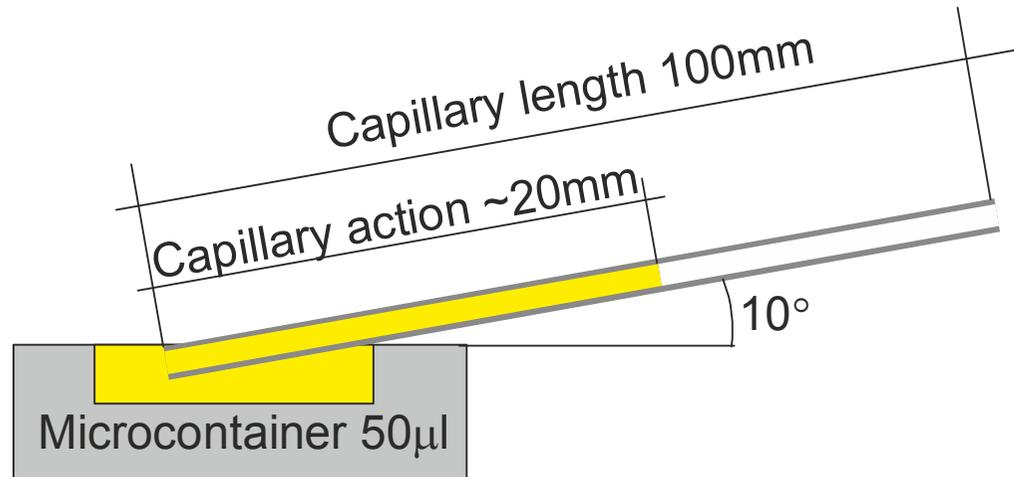
The arborization test evaluate the level of estrogen.  
The arborization test is not allways sufficient for fertility diagnose.

# Vaginal fluid arborization test of cow in functional state of fertility



Photos made with AFM S3400

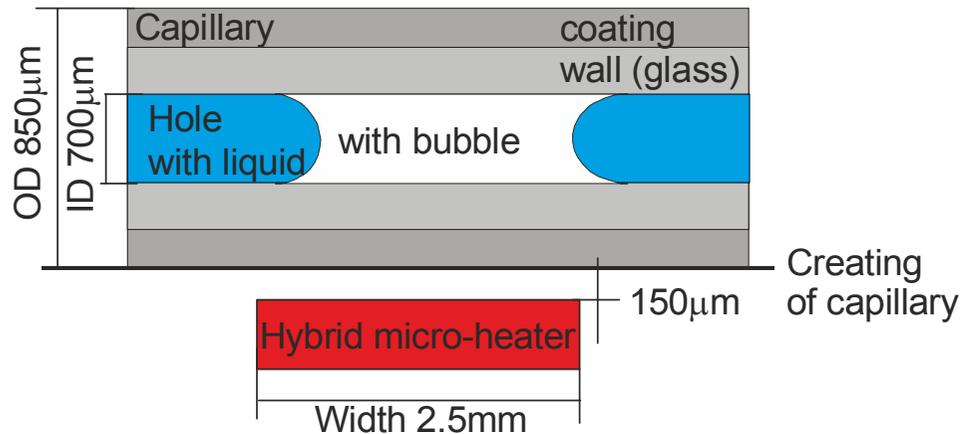
# Observation of filling of the cappilaries



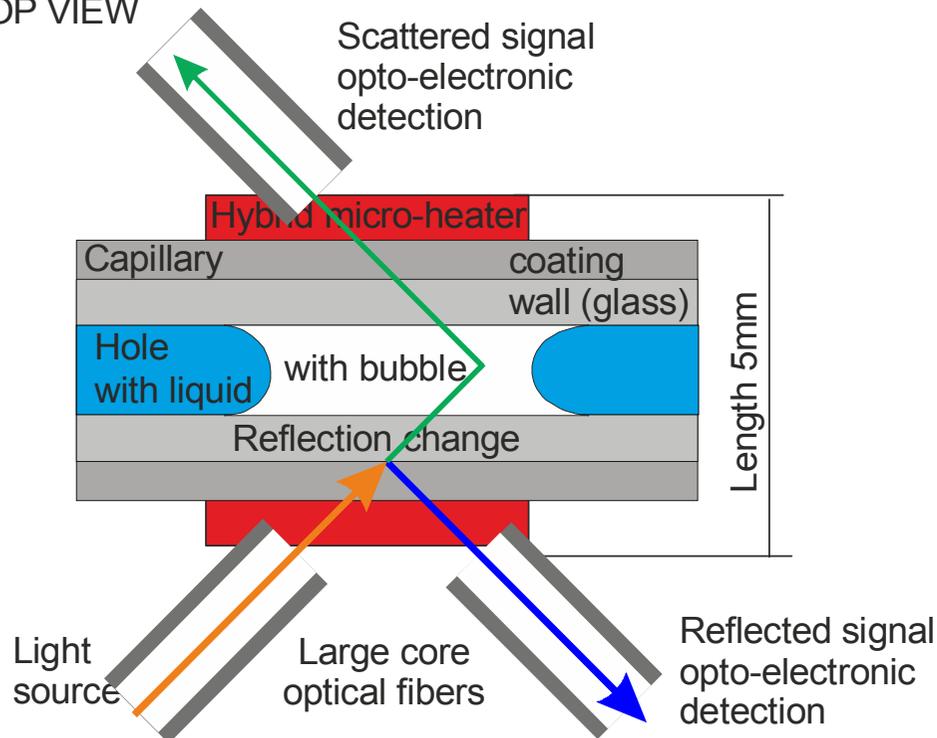
Liquid type	Capillary action [mm]	Capillary filling
Water	28	capillary action
Vaginal fluid at most fertile phase with ovulation	~20	capillary action
Vaginal fluid at most fertile phase without ovulation	0	syringe pumping working well
Vaginal fluid of cow with vaginosis	~20	capillary action
Vaginal fluid of cow with ovarian cysts	0	syringe pumping working badly

# Schematic construction of the capillary head

SIDE VIEW



TOP VIEW

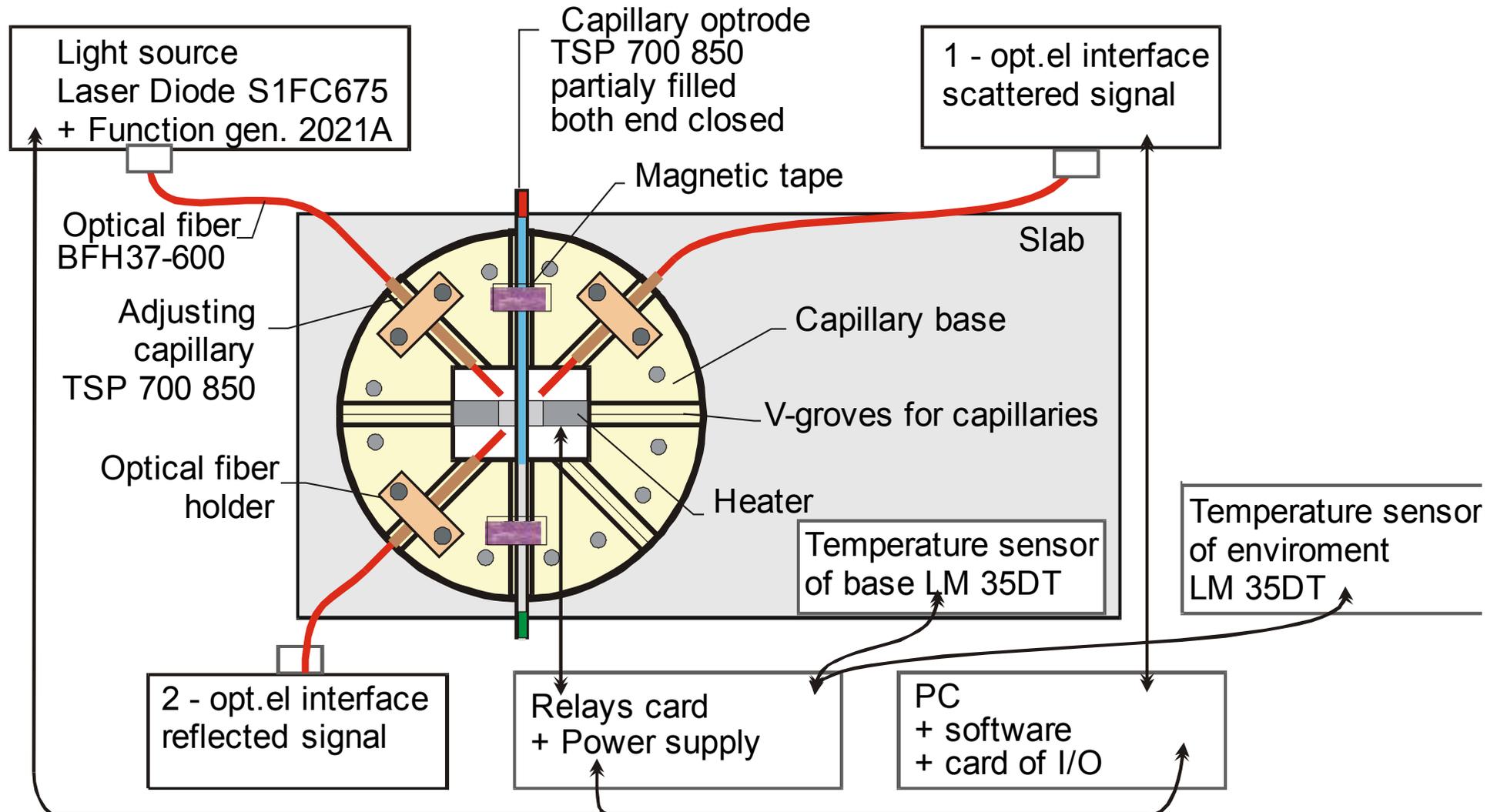


1. Working principle of proposed sensors is monitoring of optical intensity changes that take place in dynamically forced measurement cycles
2. The sensors use fiber optic capillaries in which the phase of the filling liquid changes locally to gas when forced by local heating, while the propagation of light across the capillary is monitored,
3. The sensors examine simultaneously many liquid parameters which are then processed in artificial neural networks The low cost of capillaries make their disposability practically possible.

M. Borecki, et al, „Optoelectronic Capillary Sensors in Microfluidic and Point-of-Care Instrumentation”, Sensors, (2010)

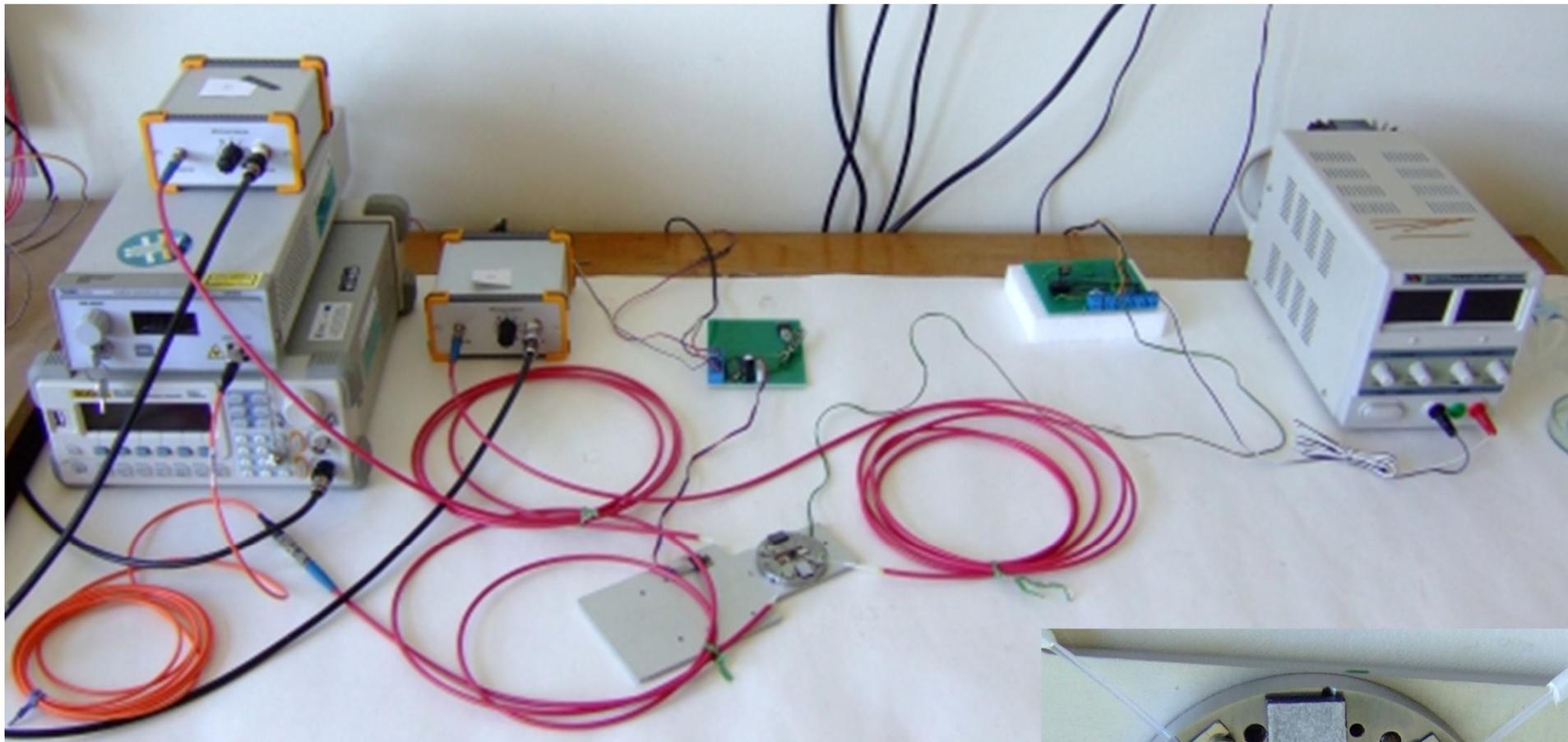
M. Borecki, et al, „Capillary microfluidic sensor for determining the most fertile period in cows”, APP, (2010).

# Schematic construction of the capillary sensor

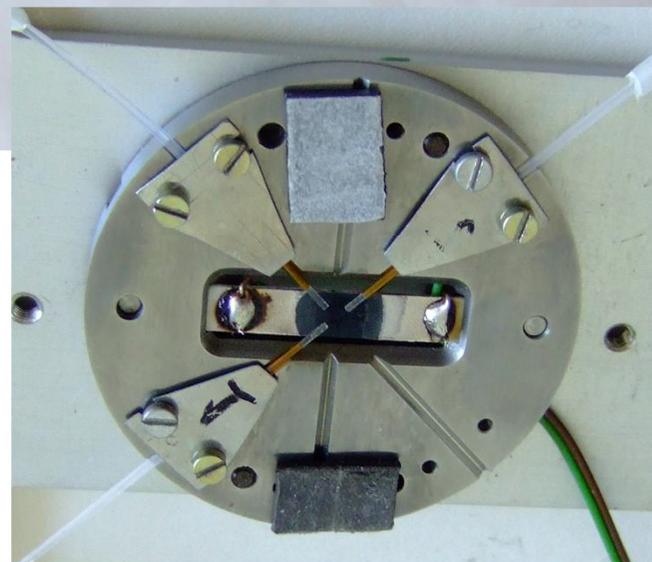


To operate the system we designed a script in DasyLab 10 with 0.1s sampling rate.

# View of the tested system

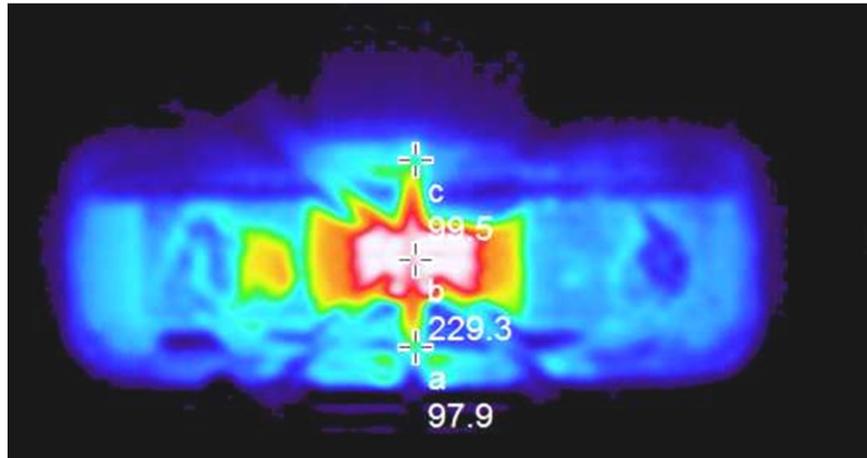


Insert: Close-up view of the sensor head

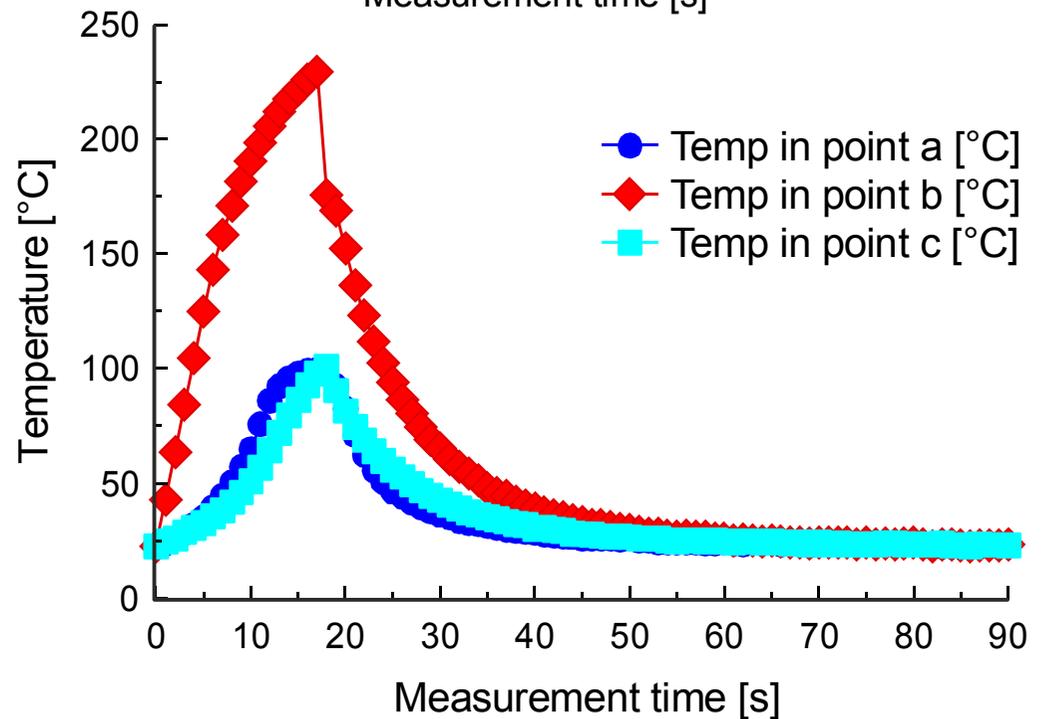
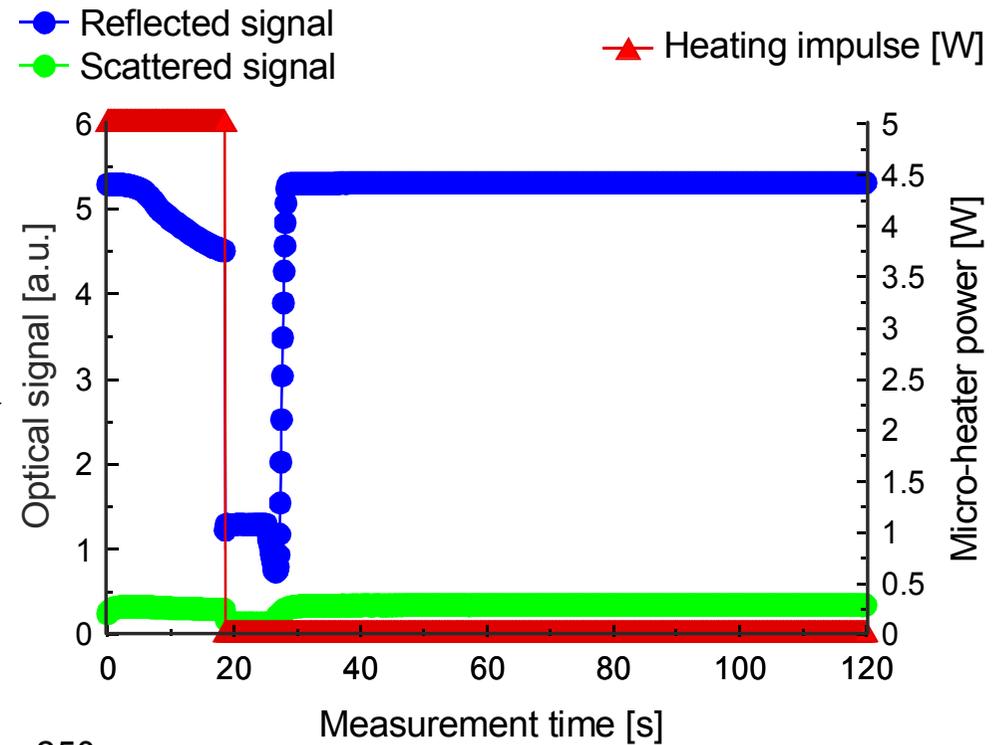


# Calibration of the sensor

Signal and temperature waveforms for calibration with DI water and heating power of 5W

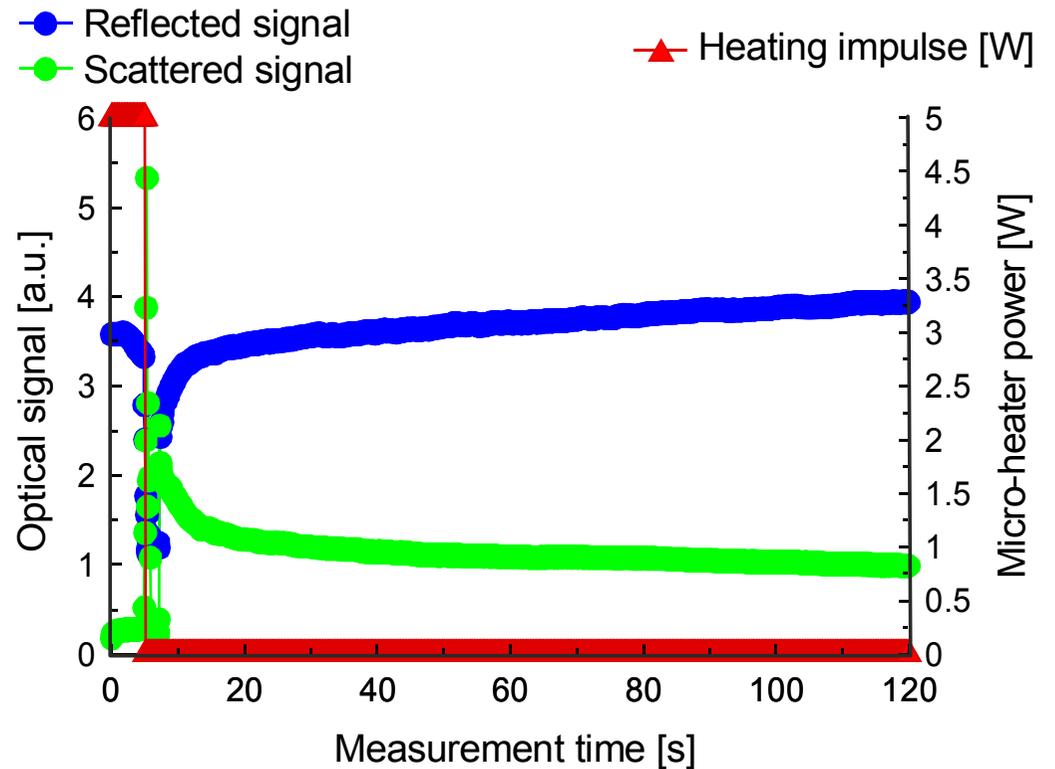


Temperature map just before boiling at 17s of the measurement procedure

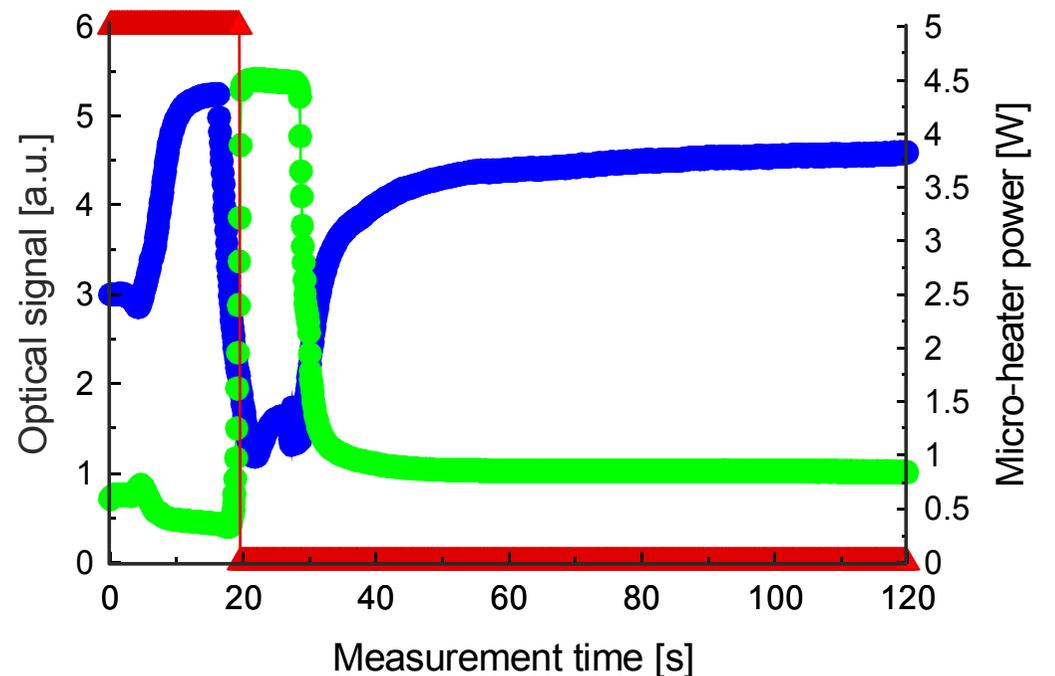


# Calibration of the sensor

Measurement procedure signals of antibody mixture in PBS - Permeabilization and Blocking Solution



Measurement procedure signals of glycerol e-coli mixture



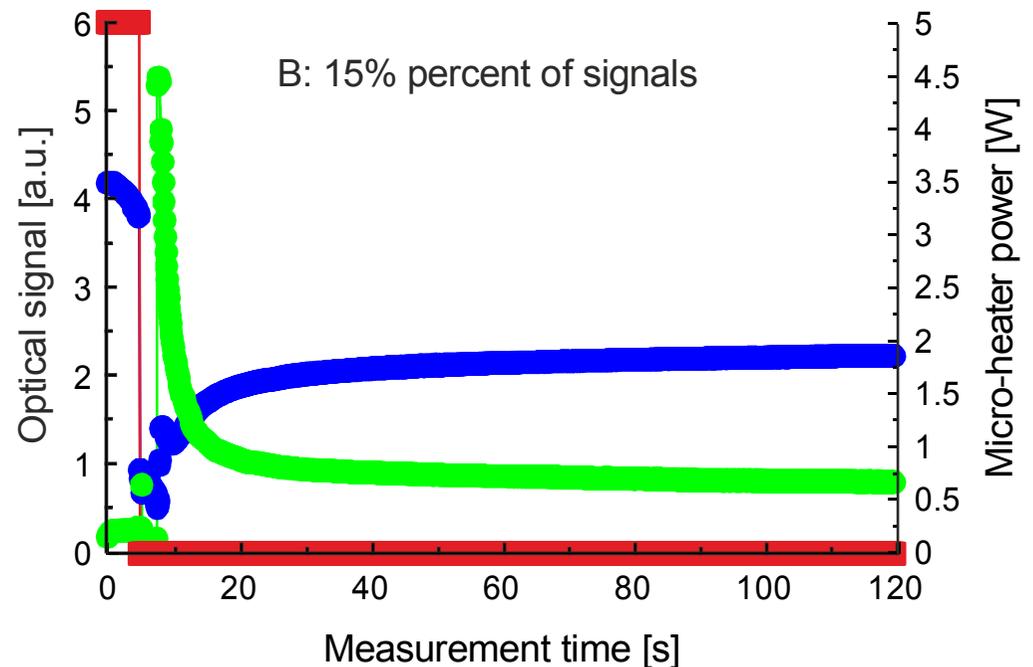
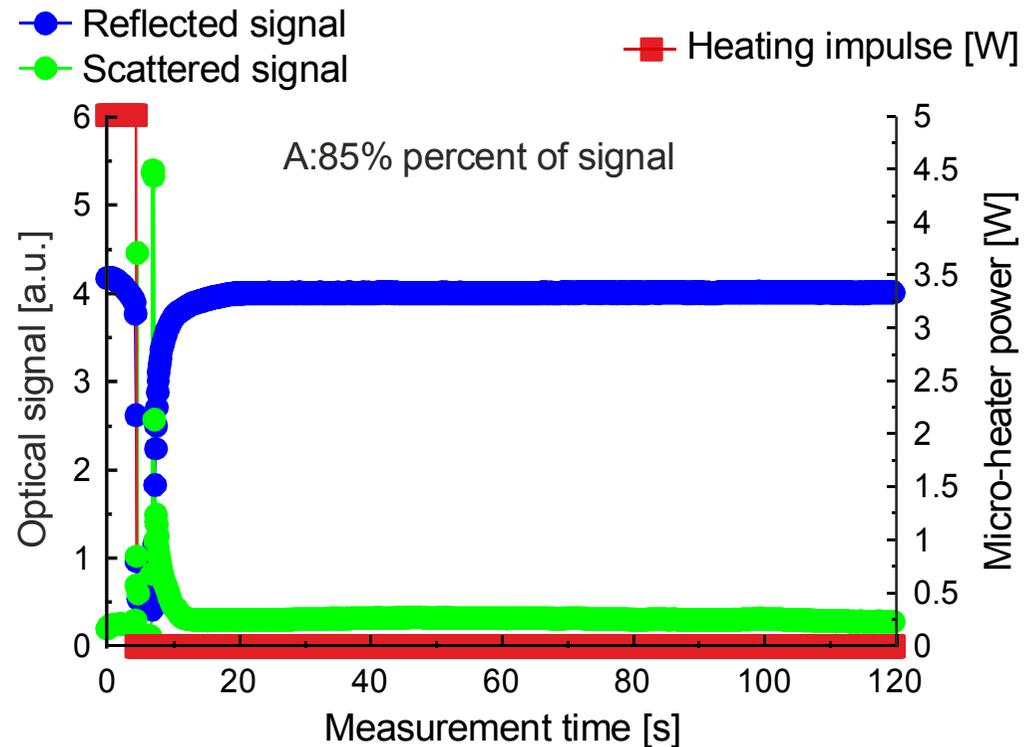
The scattered signal changes

1. could be connected with presence of antibody and bacteria,
2. may enable the detection of vaginitis.

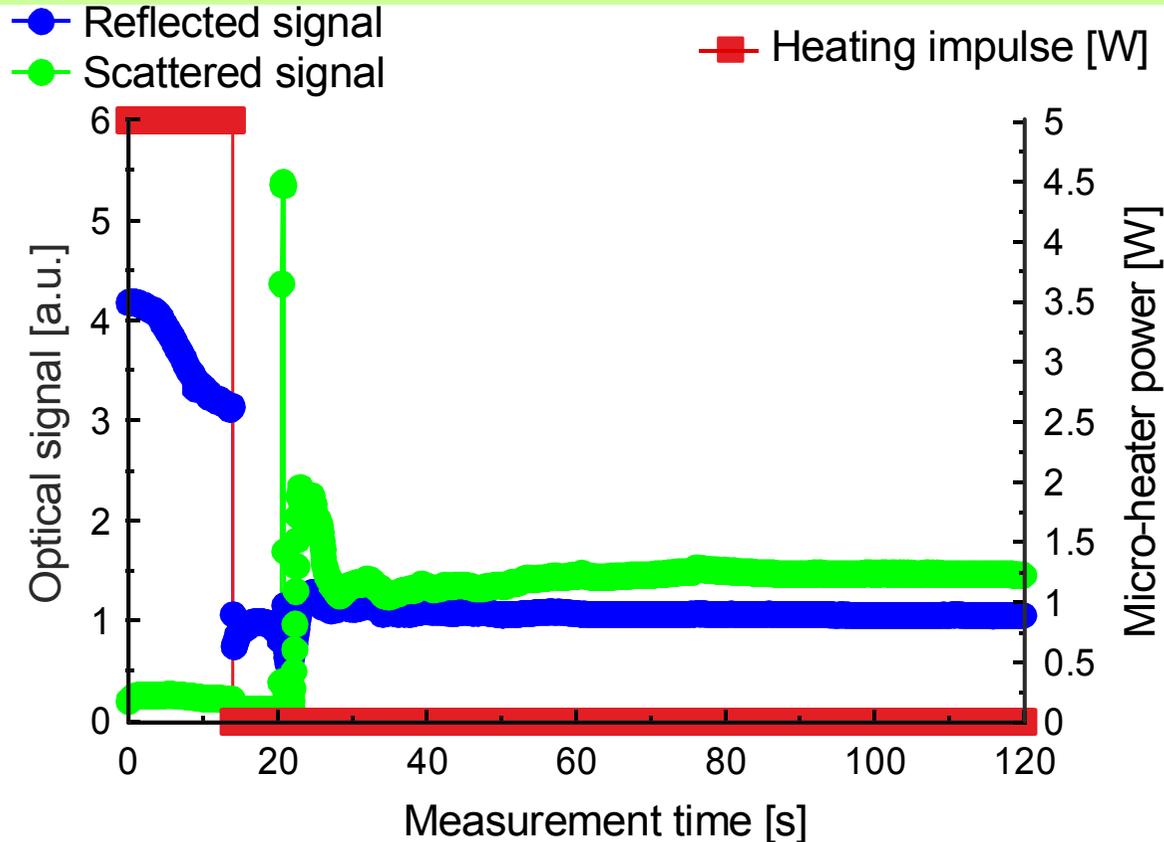
# Signals for cows classified as healthy and in oestrus state

Distinctive features:

1. Vapor phase creation time about 5 seconds.
2. Vapor phase presence for 2 seconds.
3. The moment of signals stabilization after vapor phase disappearance is at 40 second of measurement.
4. The reflected signal after stabilization is greater than 1.5 a.u.
5. The scatter signal did not exceed after stabilization the level of 1.0 a.u. but would increase over initial level correspondingly with the count of present bacteria.



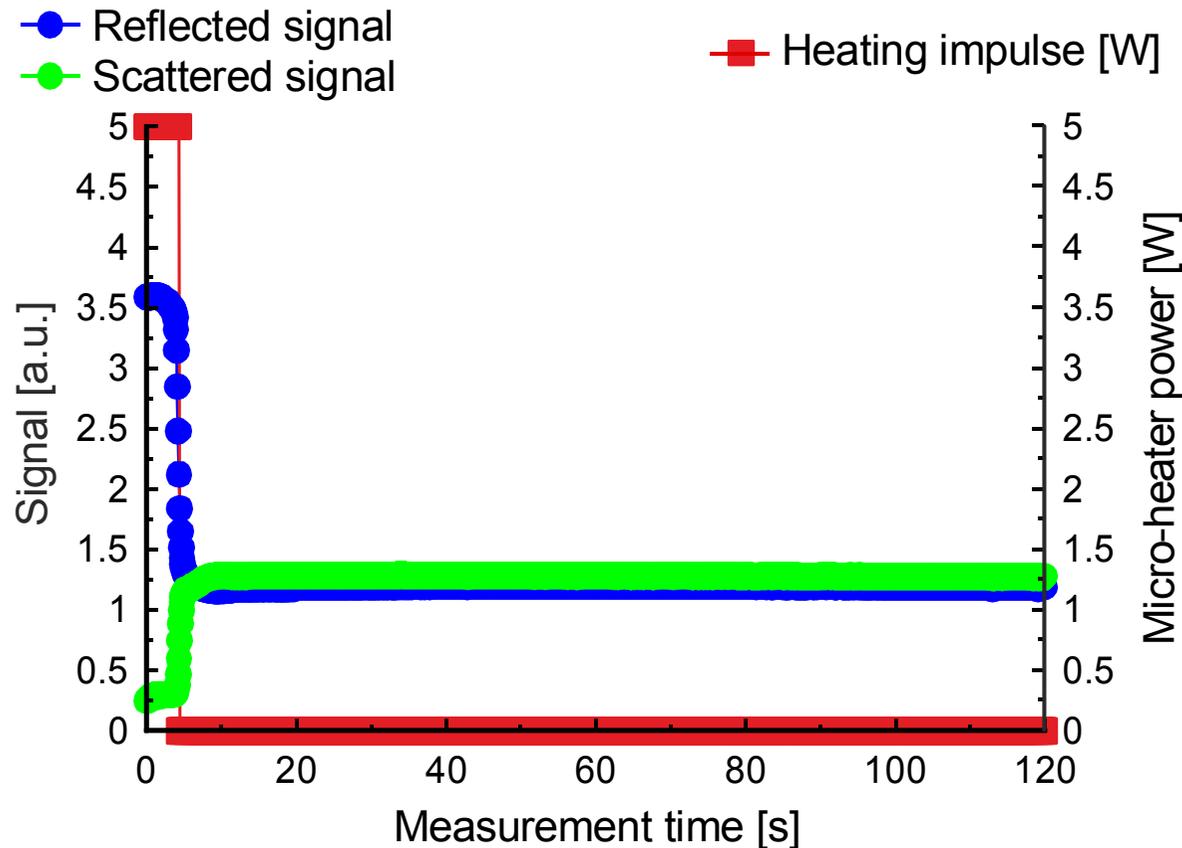
# Signals for cows classified as healthy and in late fertile phase / initial postestrus state



Distinctive features:

1. Vapor phase creation time about 12 seconds.
2. Vapor phase isn't disappearing.
3. The moment of signals stabilization is at 40 second of measurement.
4. The reflected signal after stabilization is lower than 1.5 a.u.
5. The scatter signal exceed after stabilization the level of 1.0 a.u.

# Signal of vaginal fluid of cows with vaginitis



Distinctive features:

1. Reflected signal dropping and scatter signal increasing in about 3 seconds.
2. Vapor phase does not create.
3. The reflected signal after stabilization is lower than 1.5 a.u.
4. The scatter signal exceed after stabilization the level of 1.0 a.u.

# ANN for functional state of fertility classification

1. We examined the multilayer perceptron for classification of measured signals.
2. We assumed two output information levels: a) healthy/unhealthy, b) ready for reproduction/ not ready for reproduction.
3. Our ANN had 8 inputs, 2 outputs and 4 hidden layers in which the perception used the sigmoid activation function and one hidden input with constant -1 value.

Input	Input contribution [%]	
	<i>Output for cows ready for reproduction</i>	<i>Output for cows in functional state</i>
Initial level of reflected signal	14	1
Time of gas phase creation	35	83
Time of gas phase lasting	30	11
Minimal level of reflected signal	8	3
End level of reflected signal	2	0.5
Initial level of scattered signal	5	0.5
Maximum level of scattered signal	4	0.5
End level of scattered signal	2	0.5

# ANN for functional state of fertility classification

ANN training gave:

1. 0.986 correlation coefficient in the training set,
  - the output responsible for healthy state correlation coefficient of 0.999,
  - the output responsible for oestrus correlation coefficient of 0.987.
2. 5% RMS error of outputs when cases from monitored cows but not contained in the training set were analyzed.

For the worst case test of the network output.

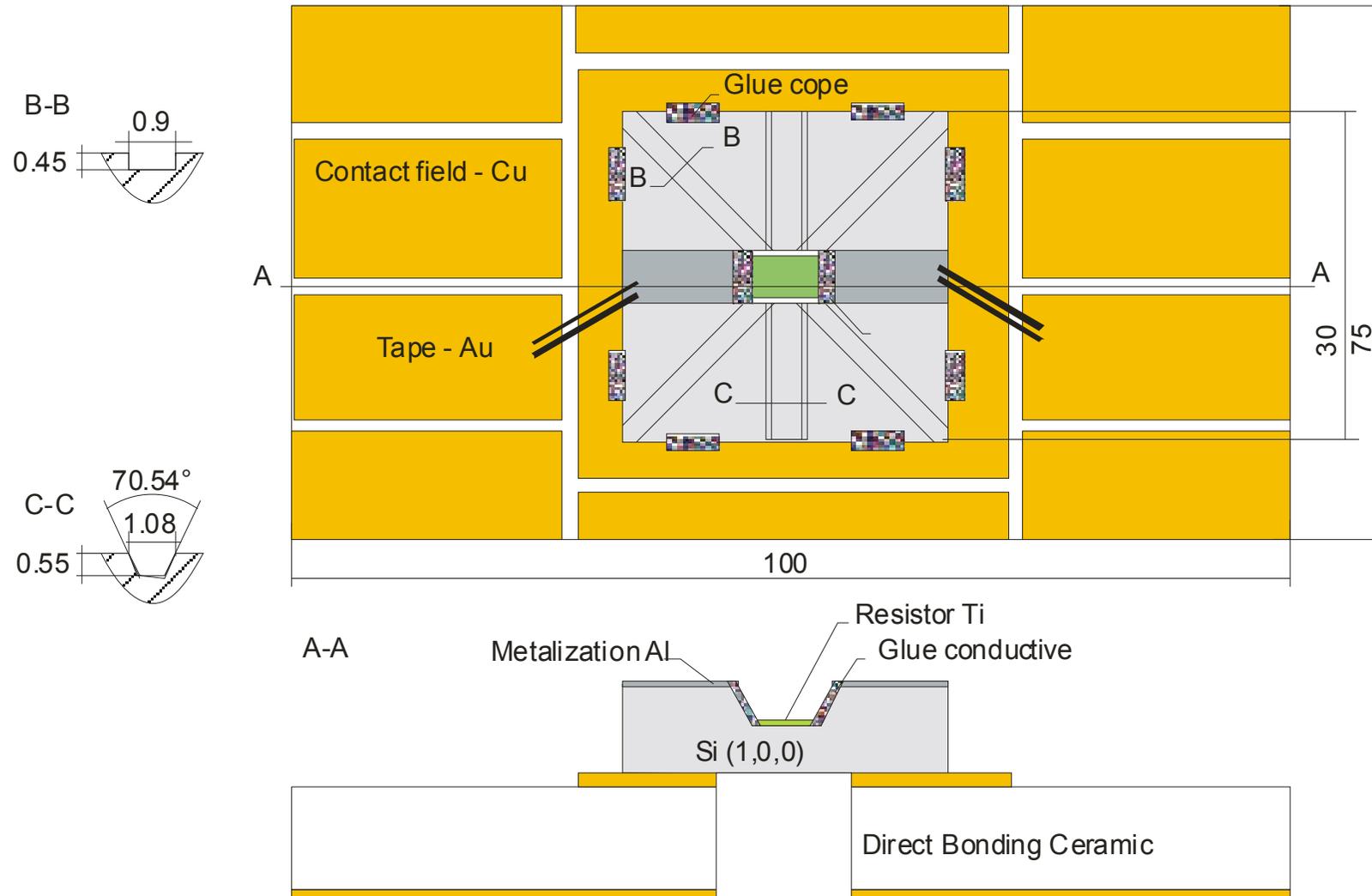
1. We test vaginal fluid of cow that is not included in training set and has first oestrus after miscarriage.
  - The third oestrus state after miscarriage is normally consider as fertile - therefore, a question arise of mentioned cow healthy and fertile state.
2. The error of most fertile state was 21%
3. The error of functional state was 15%
4. Therefore, the 5% of outputs tolerance of functional state of fertility classification seems reasonable.

# Integration idea

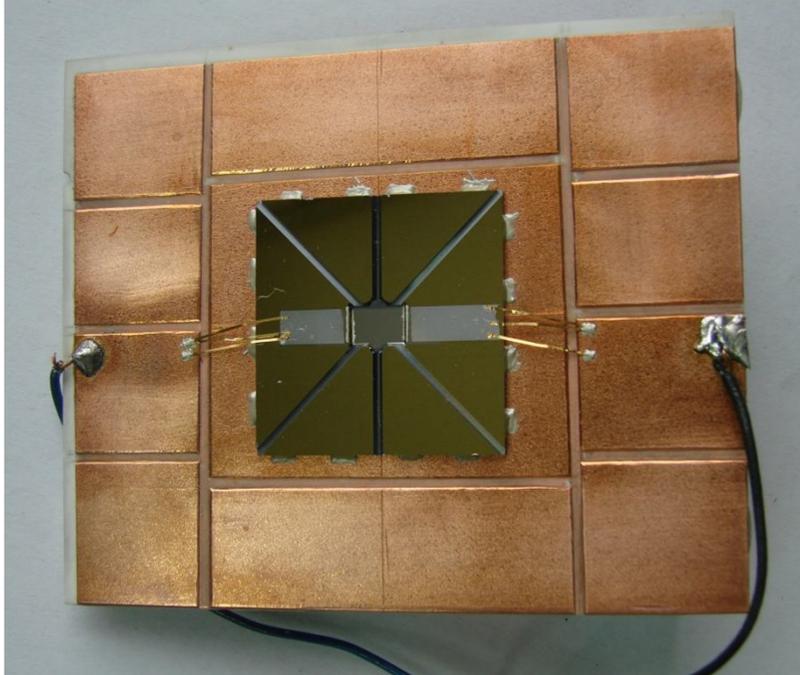
- The fibers and capillary outer diameter is about  $800\mu\text{m}$ .
  - The silicon wafer with the thickness of  $900\mu\text{m}$  is required.

The wafer processing available are:

- IC technologies,
- Micromachining,
- Hybrid technologies.



# Capillary basis with use of Si structure

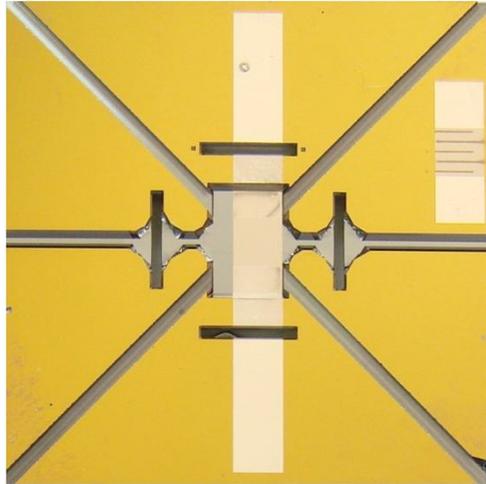


- The heater power required for water boiling in capillary is 25W.
- The heat transfer balance have to be improved.
- The high temperature conductive glue have to be withdraw

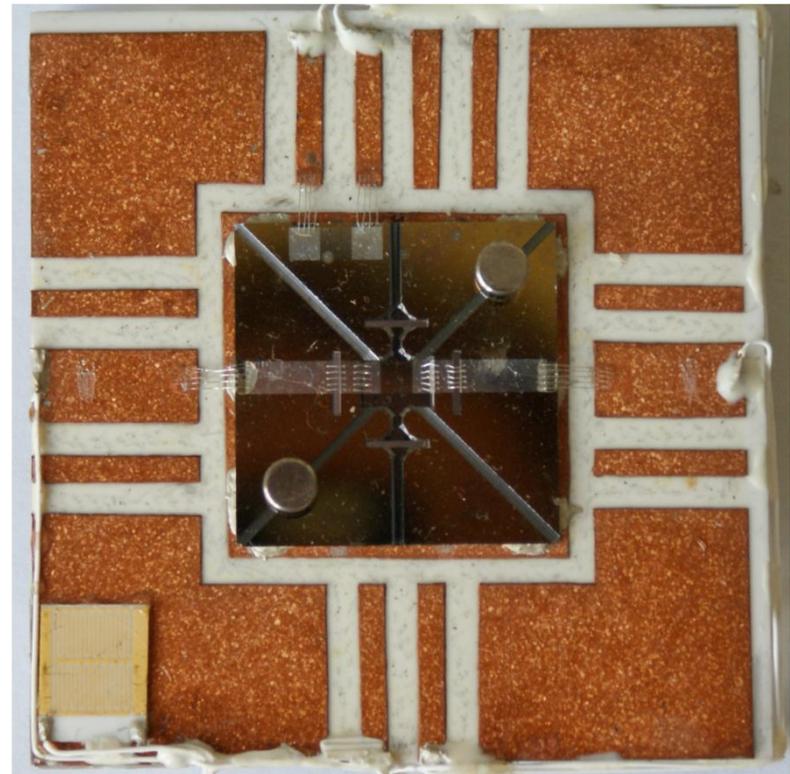
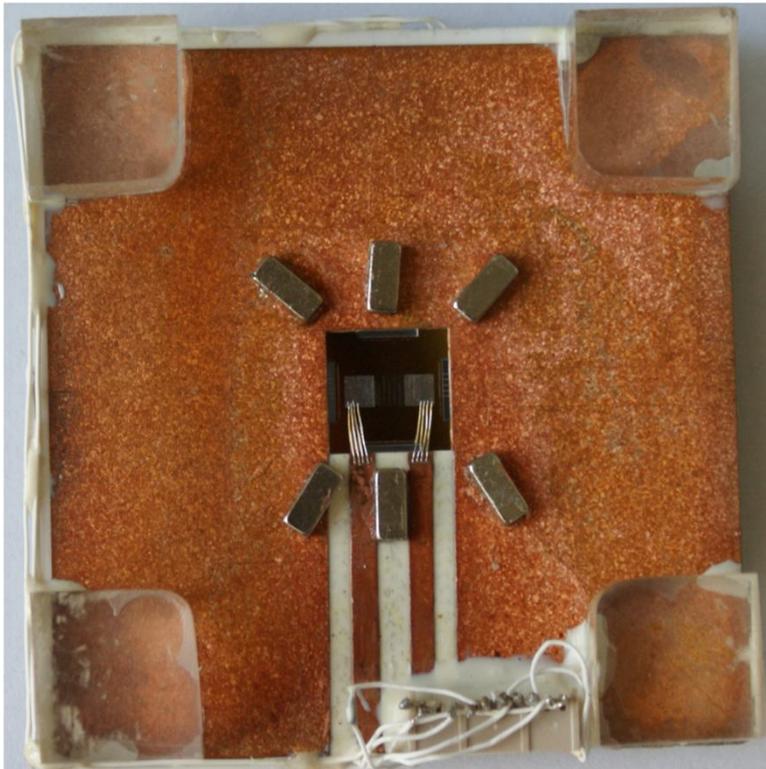


- The screw holders of fiber and capillary are not comfortable in use.
- The magnetic holders work better

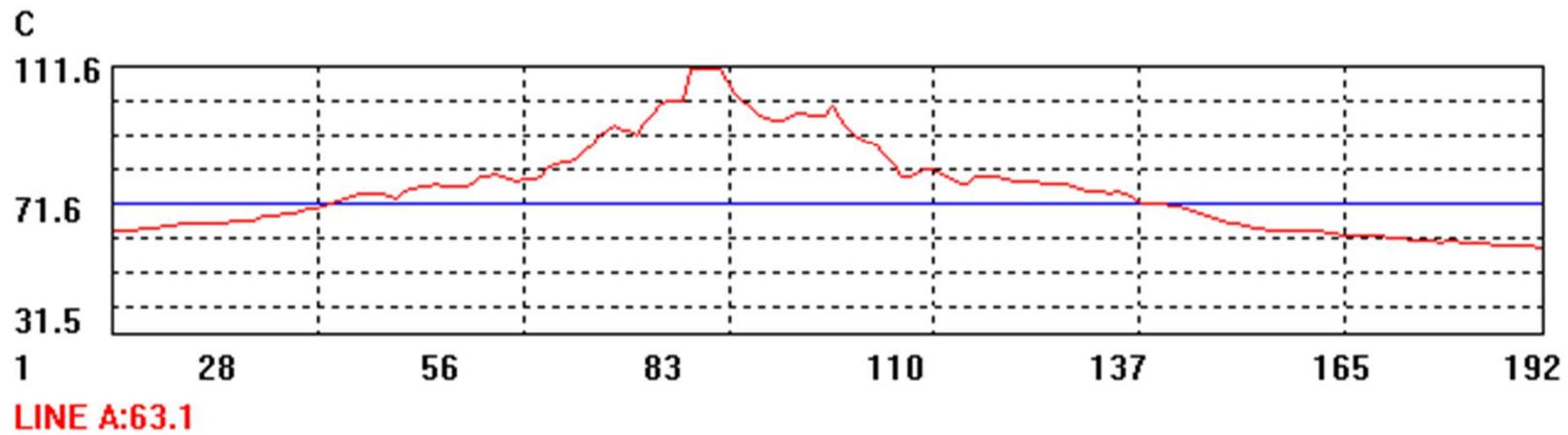
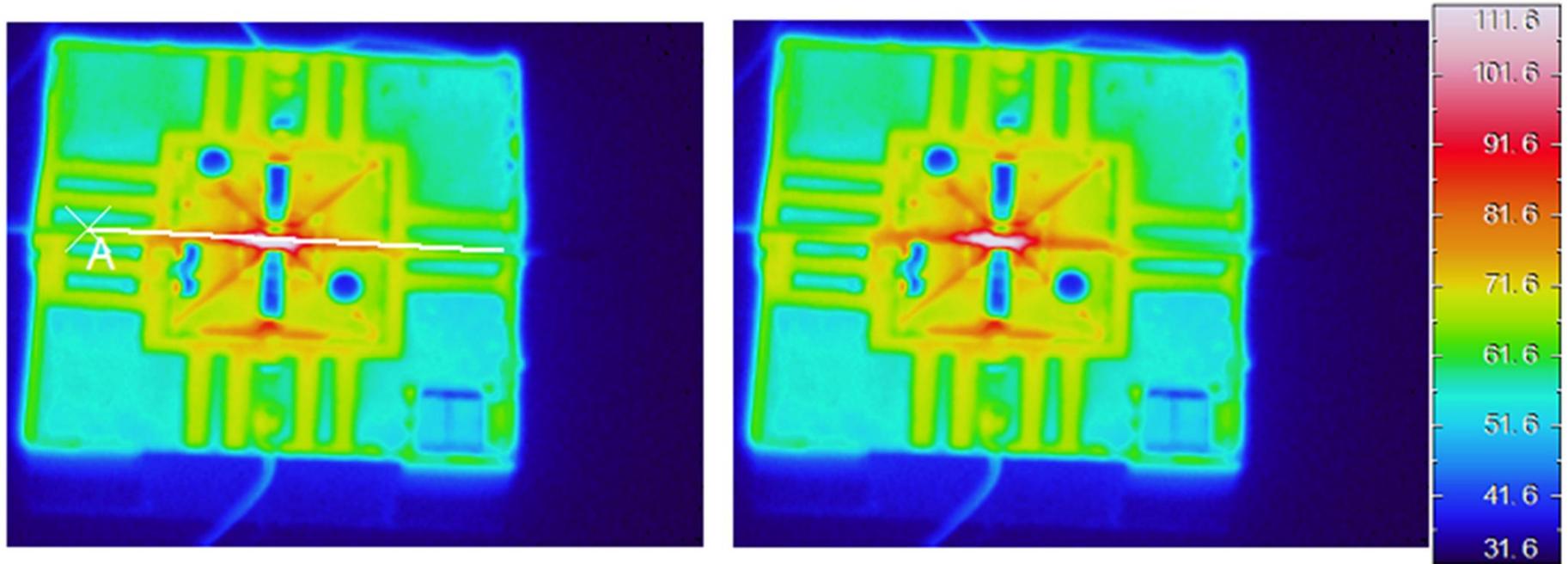
# Improved construction



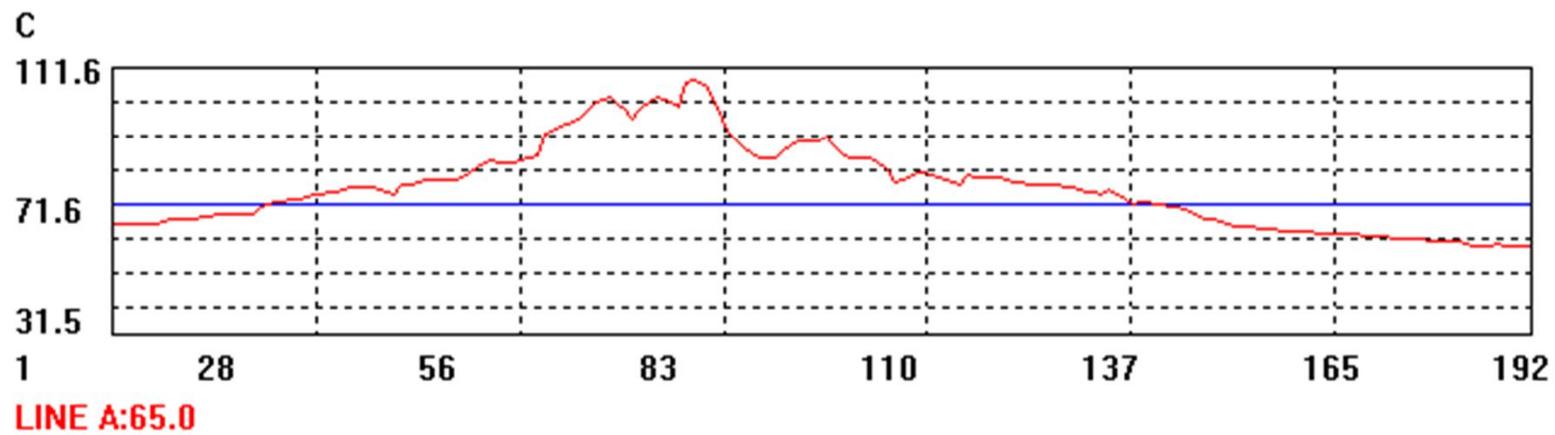
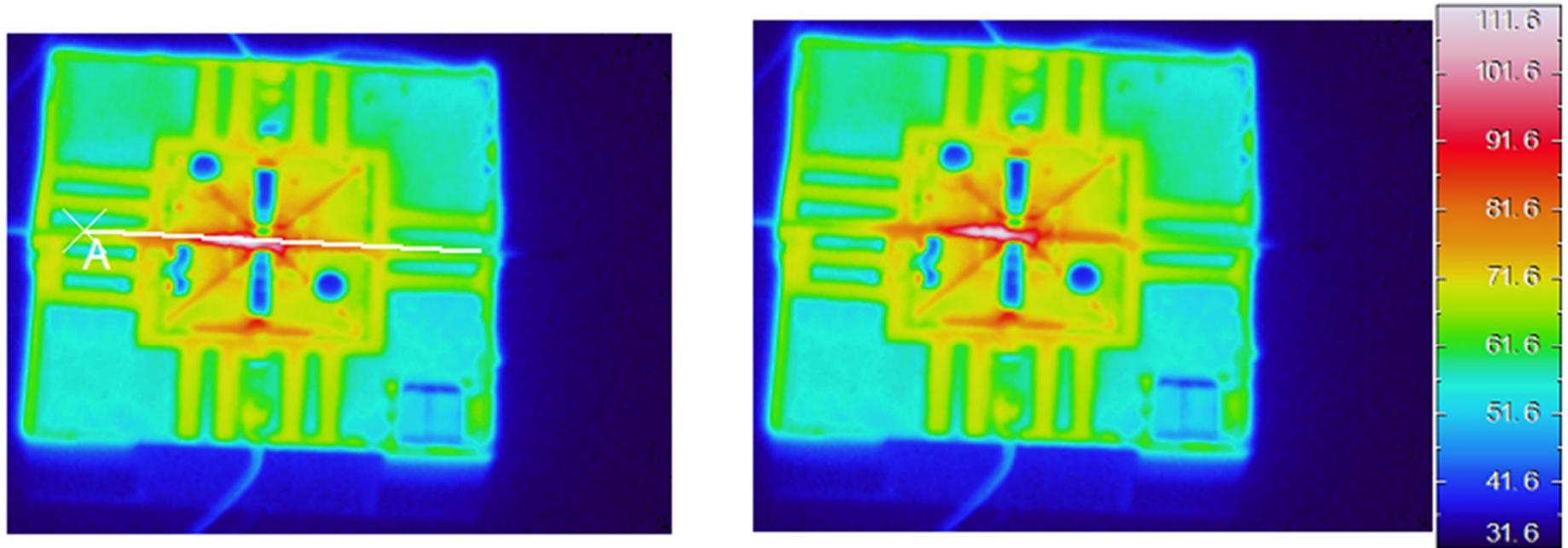
1. Four side heater thermal „air isolation”
  - 16W required for water boiling in capillary
2. Three integrated resistance sensors of temperature
3. Magnetic holders of fibers and capillary



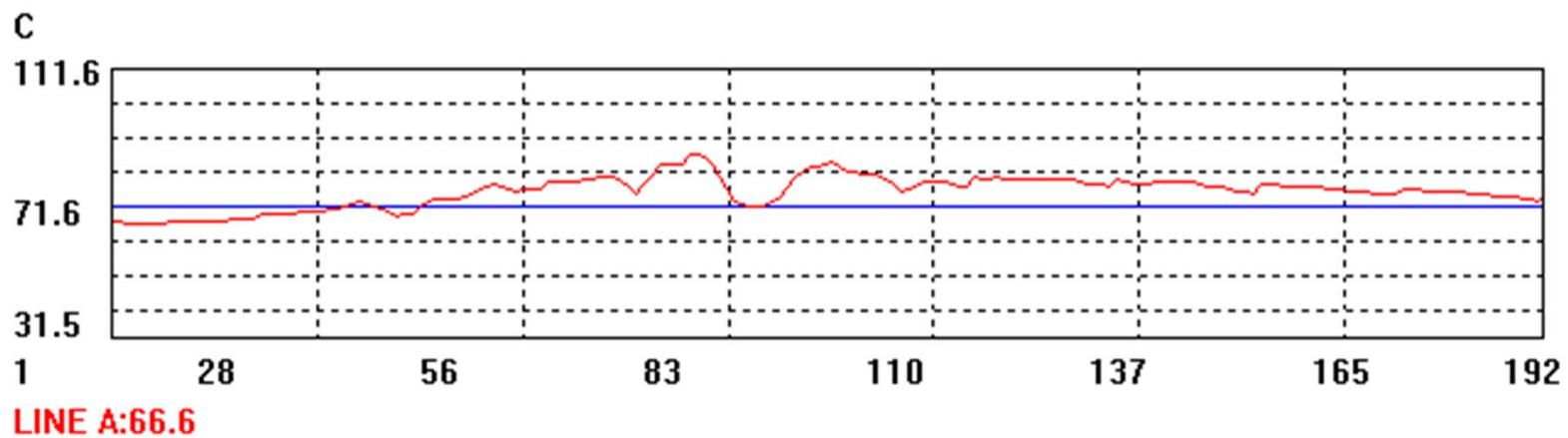
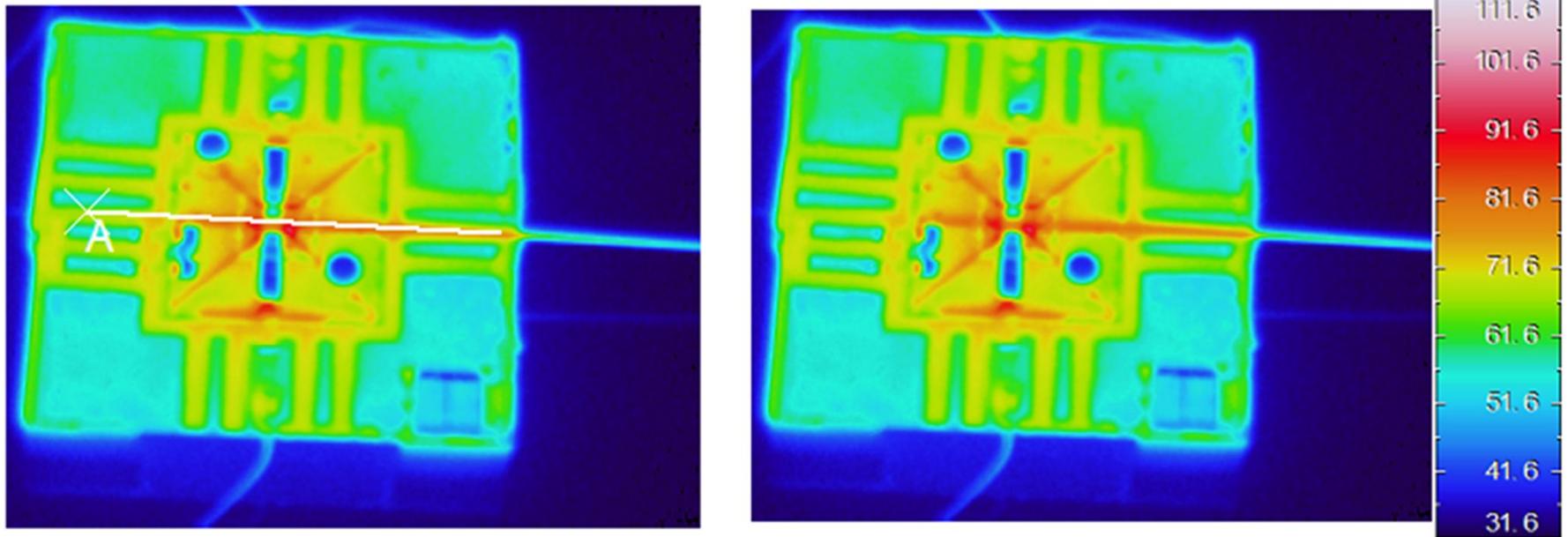
# Water boiling – begining 56.20sec



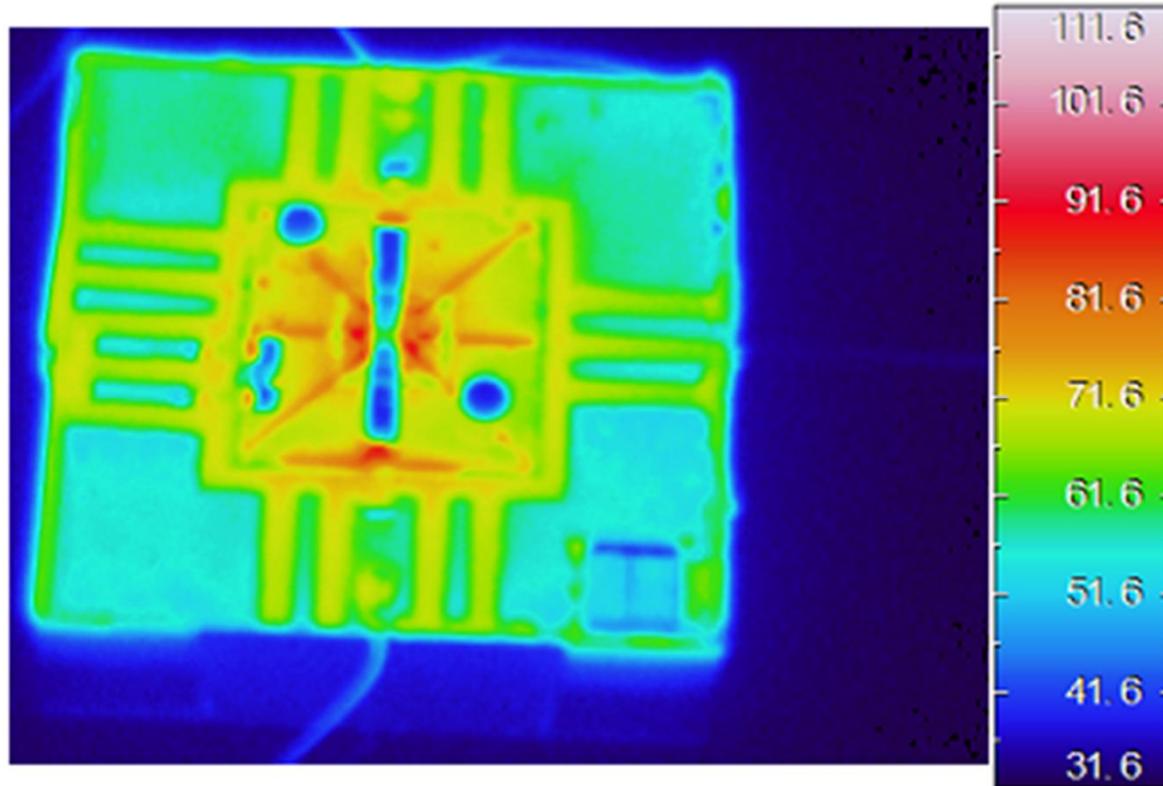
# Water boiling – first displacement of vapor 57.0sec



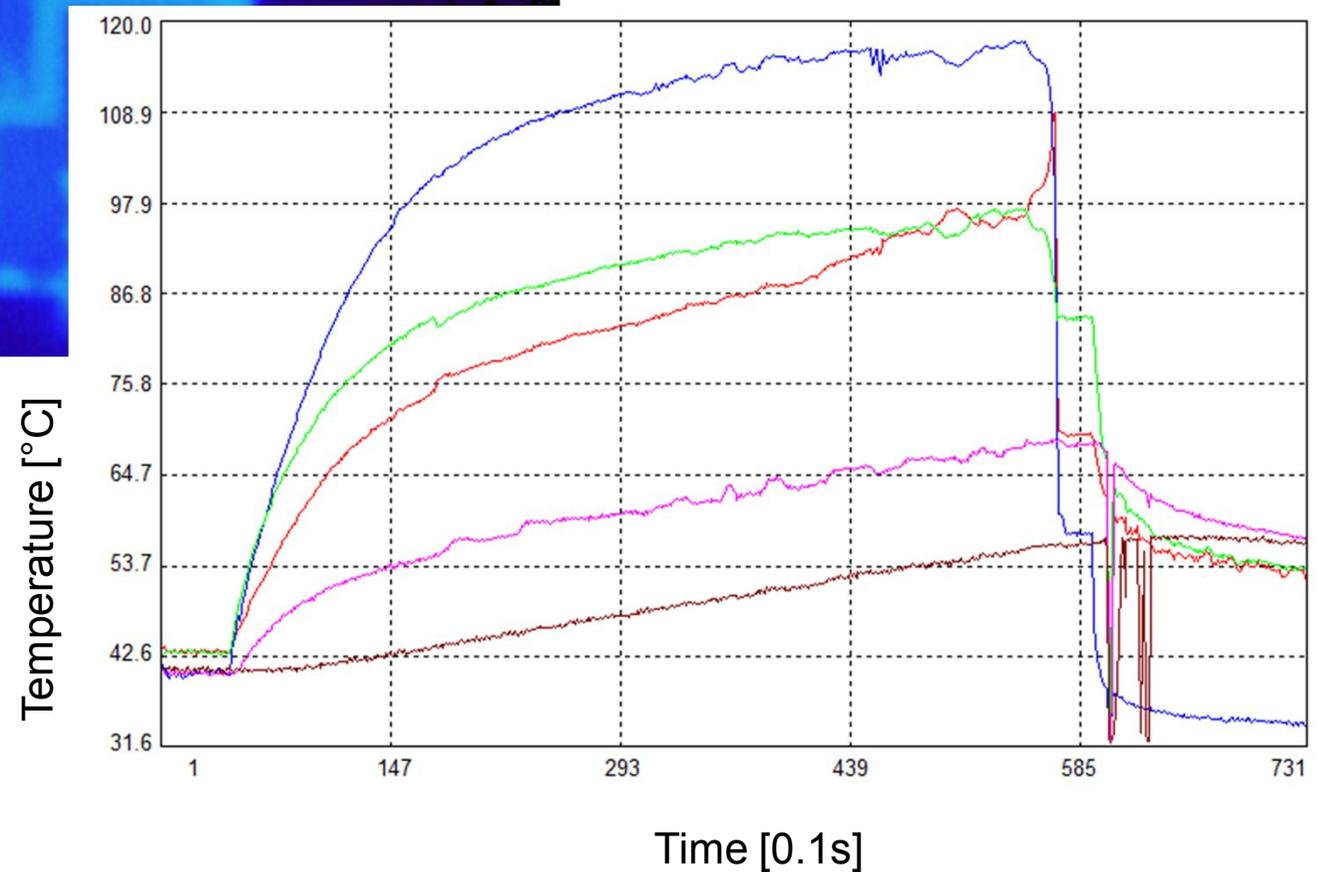
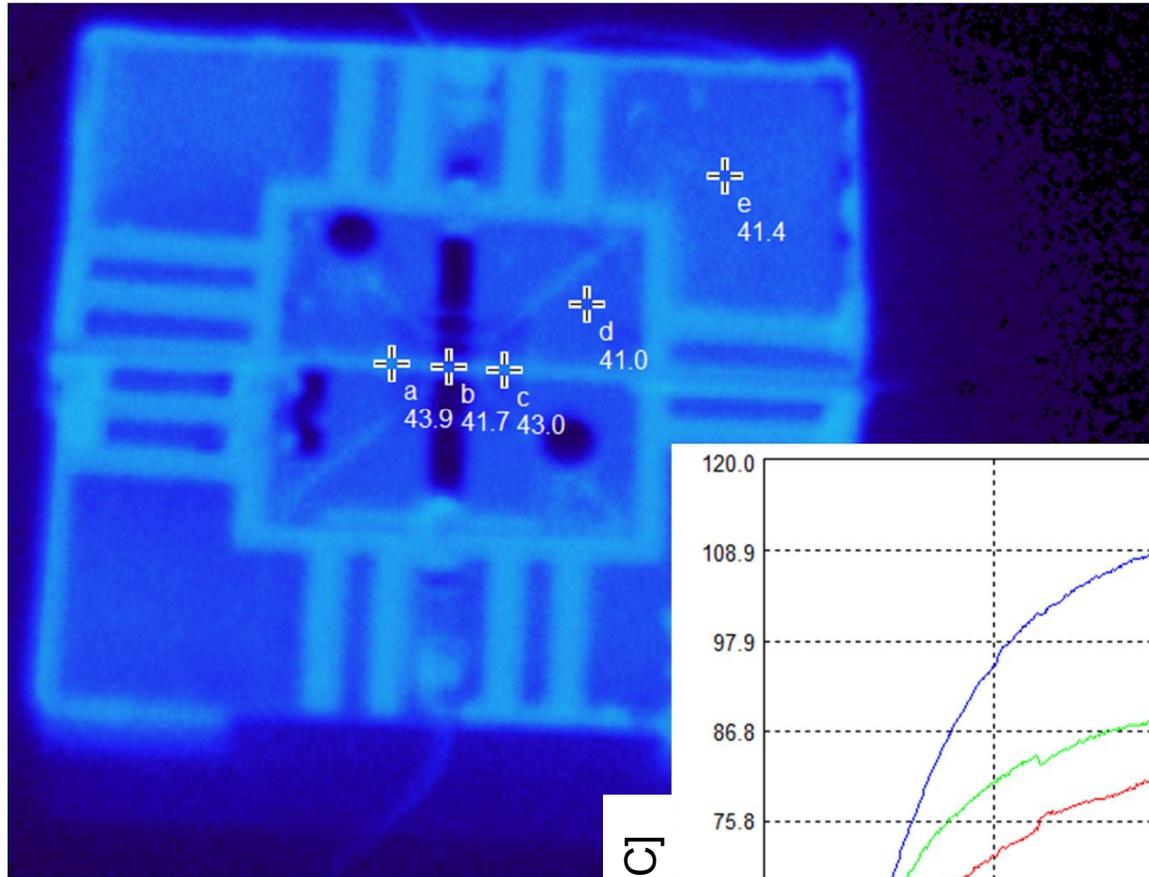
# Water boiling—second displacement of vapor 57.01sec micro-heater switch-off



# Head cooling - 57.02sec



# Integrated structure examination – water boiling



# Conclusion and future work

1. We have proved that the fiber optic capillary sensors with artificial network analysis can provide information on cow oestrus state with a sensitivity and specificity superior to other existing methods and in addition provide information as to the presence of cow vaginitis.
2. The results of the measured signals analysis of ANN training showed a correlation of 0.987 for oestrus and 0.986 for the classification of functional state of fertile phase.
3. The proposed sensor introduced automatically precise information of possible vaginitis which can be of practical usefulness.
  - We showed that the information on vaginitis was obtained mainly by establishing the presence of antibodies in the vaginal fluid. These antibodies are normally transparent in visible light in standard situations.
  - The capillary filling capability can act as an indicator of the most fertile state of cow oestrus.
4. Therefore, we conclude that the proposed construction may be in future the base of commercially marketable instruments.

Thank you for attention